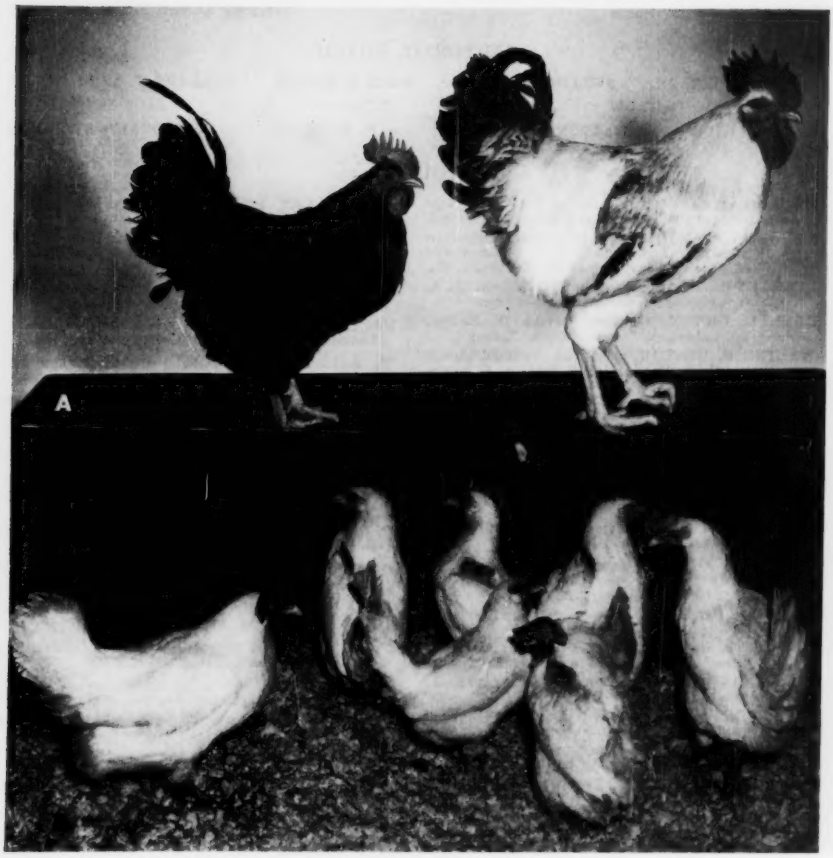
The Journal of HEREDITY



SEX-LINKED DWARFS
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Tufted Mice

Cotton Twins

Cereal Hybrids

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The American Genetic Association

An incorporated organization devoted to promoting a knowledge of the laws of heredity and their application to the improvement of plants, animals, and human racial stocks

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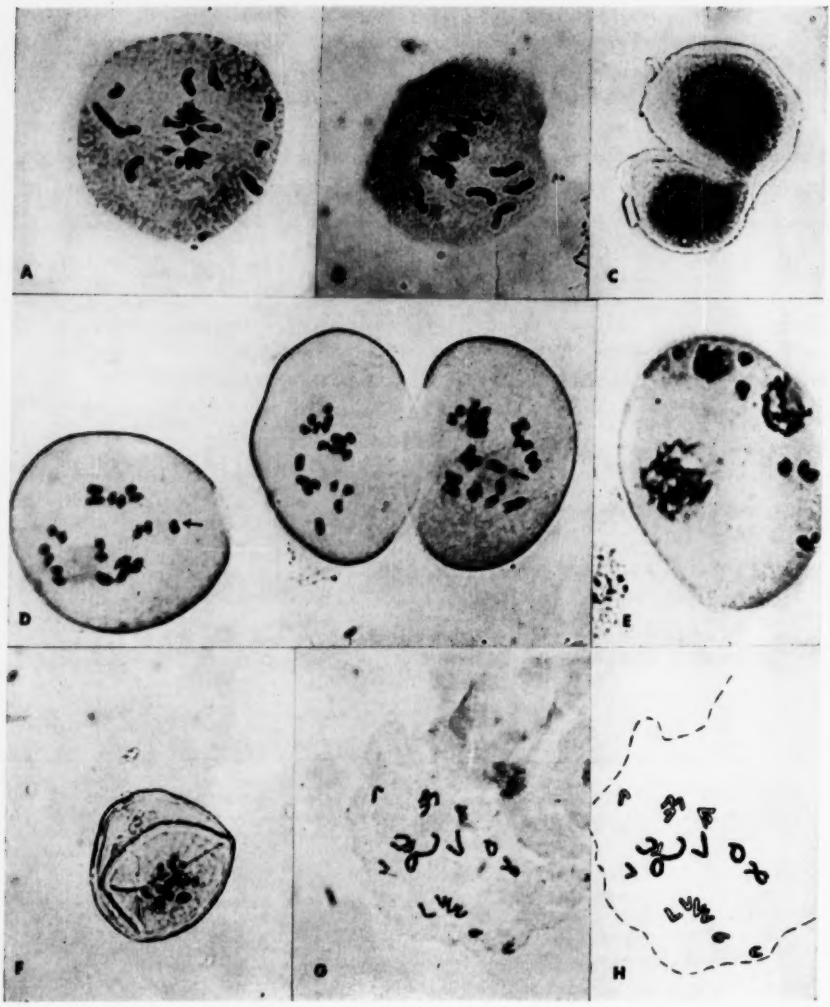

MEIOSIS OF *HORDEUM JUBATUM* × *SECALE CEREALE* VAR. ANTELOPE

Figure 1

A—Metaphase I: six *Hordeum* bivalents and nine univalents of which seven are large *Secale* chromosomes. *B*—Metaphase I: seven *Hordeum* bivalents and seven *Secale* univalents. *C*—Diad at interphase, showing separation of daughter cells. *D*—Prometaphase II: cells rounded off and single; chromosomes split into their chromatids, except a few that have already split in anaphase I (arrow). Note nucleoli. *E*—A later stage: cells with groups of chromosomes forming nuclei and with some remotely scattered chromosomes. *F*—Degenerating cell with chromosomes, split into their chromatids, still visible. *G* and *H*—Photomicrograph and photodrawing of anaphase II cell: 14 *Hordeum* and six *Secale* chromosomes (black).

INTERGENERIC HYBRIDS BETWEEN HORDEUM JUBATUM L. AND SECALE CEREALE L.

E. B. Wagenaar*

In phylogenetic studies, interspecific and intergeneric hybridization followed by a study on the bivalent formation at meiosis of the hybrid is a common method to obtain information concerning homology between the chromosomes of parental species. Within the tribe *Hordeae*, much work of this kind has been done on the genera belonging to the subtribe *Triticinae*. Relatively little is known, however, on genera outside this subtribe, partly due to the difficulty in obtaining the hybrids of several interspecific and intergeneric species combinations. This is the situation in the genus *Hordeum* where relatively few hybrids have so far been reported. One such hybrid, of special interest here, is that between *Hordeum jubatum* L. ($2n = 28$) and *Secale cereale* L. ($2n = 14$). This hybrid was previously obtained by Brink *et al.*[4], who used the method of embryo culture. Without embryo culture this and similar crosses have proven unsuccessful[24,25,26,30], since the young hybrid caryopses degenerate within 14 days after fertilization[3,4,7,30].

In their *H. jubatum* × *S. cereale* hybrid, which had a somatic number of 21 chromosomes, Brink and his co-workers observed five loosely associated bivalents at diakinesis. They were, however, unable to determine to which genomes the chromosomes that participated in the bivalent formation belonged, since all chromosomes appeared to be alike in size and form.

In this respect it is of interest to note that in other hybrids involving *S. cereale*, von Berg[2] (*Aegilops triuncialis* × *S. cereale*), and Stebbins and Pun[29] (*S. cereale* × *Agropyron intermedium*) were able to distinguish the seven large rye chromosomes from the others. This characteristic enabled them to conclude that bivalent formation observed in their hybrids was due to autosyndetic pairing of the *Aegilops* and *Agropyron* chromosomes, respectively. On the other hand, Kagawa and Chizaki[13], and Gaul[12] had occasional difficulties in distinguishing the larger rye chromosomes at meiosis in their hybrids with *S. cereale*. They observed from five to eight large chromosomes in the pollen-mother-cells.

In the present paper the cytological features of two hybrids between *H. jubatum* and *S. cereale*, also obtained by embryo culture, will be described and discussed. The attempts to produce these hybrids were undertaken as part of a hybridization program for the purpose of cytological studies and breeding work. In the light of the results of von Berg[2] and Stebbins and Pun[29], it was hoped that a critical study on the meiosis of this hybrid would reveal differences in size between the chromosomes of *Hordeum* and *Secale*. This then would facilitate the identification of the chromosomes involved in bivalent formation, as observed by Brink *et al.*[4], and thus permit an estimate of the degree of homology existing among the three genomes

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concerned.

Materials and Methods

H. jubatum plants used in the study were grown from seeds collected at Edmonton, Alberta. The three rye varieties—Antelope, Petkus and Sangaste—were used as the pollen parents.

Ten to 12 days after pollination the hybrid embryos were transferred to a nutrient medium. No attempt was made to isolate the embryos completely from the immature caryopses. The tip containing the embryo was carefully cut off, immersed in calcium hypochlorite, rinsed in sterile water, and placed on the surface of the nutrient medium. This simple method avoids all possibility of damage to the embryo and gives satisfactory results.

The medium consisted of commercial Difco orchid agar to which the following were added: Difco yeast extract (0.04 g./1.), casein hydrolysate (1 g./1.) and tryptophane (0.04 g./1.). Test tubes with screw caps were used as culture containers, and the embryos were incubated at room temperature under high humidity.

For the cytological studies of the pollen-mother-cells, whole immature spikes were fixed in Carnoy's solution A (six parts alcohol: three parts chloroform: one part acetic acid). Squash preparations were made in aceto-carmine.

Results of Embryo Culture

Of the 155 cultured embryos of this cross 20 were lost as a result of contamination. Six of the remainder germinated, of which only two formed roots as well as coleoptiles. These two showed slow initial growth after potting but developed later into healthy vigorous plants. The two hybrids were descended from the same *H. jubatum* plant; the rye variety used as pollen source was Antelope (No. 33) in one case and Sangaste (No. 134) in the other.

Some Characteristics of the Hybrids

Some of the morphological features of the hybrids are similar to those of *H. jubatum*, others to those of *S. cereale*. The majority of the features are intermediate between those of the parents. Two connected characteristics, however, the lengths of sheaths and leaf-blades show a transgressive effect, since both appear shorter than in the smallest parent, *H. jubatum*. Culm lengths, in contrast, are intermediate. As a result, smaller portions of the stems of the hybrid are covered by the sheaths than in the parents.

The spikes appear superficially similar to those of the rye parents. A closer examination of the spikelets, however, reveals that the floral organs are also more or less intermediate in size and structure to those of the parental species. Usually the spikelets are composed of two florets, although one floret per spikelet is common. Most frequently there are two glumes per spilelet in the hybrid; some extra small glumes are rarely observed between the two florets of the hybrid spikelet. The basal hairs of the spikelets, characteristic of rye, are entirely lacking in the hybrid.

The hybrids are completely sterile. Although a total of 30 clones were treated with colchicine, the attempts failed to produce the amphiploid. The hybrids proved to be perennials. They thrive well in the climatic conditions of Western Canada and are thus very winter-hardy. If it is possible to obtain the amphiploid, they may be of value to the forage crop breeder.

Cytological Observations

In the two parental species *H. jubatum* and *S. cereale*, meiosis was normal; 14 and seven bivalents, respectively, were regularly formed. It is noteworthy that multivalents were never seen in either parent.

The hybrid between these two species had 21 chromosomes, as expected. In the pollen-mother-cells, seven large (*Secale*) and 14 smaller (*Hordeum*) chromosomes were readily observed. This observation is in contrast to that of Brink *et al.*[4], who found no difference in size among the chromosomes in their hybrid.

Metaphase I of the hybrid which had the variety Antelope as the rye parent (No. 33) usually showed three to eight bivalents. Cells with five, six and seven bivalents occurred most frequently (Table I). The average number for

all 167 cells studied was 5.9 bivalents per cell. Bivalent formation occurred almost exclusively between the small chromosomes indicating autosyndesis of the *H. jubatum* chromosomes. The univalents usually consisted of seven large, thick rye chromosomes and a number of smaller *H. jubatum* chromosomes (Figure 1*A* and *B*), the number depending on the amount of bivalent formation. Forty-one percent of the bivalents were rod bivalents (with one chiasma) and 59 percent were closed or ring bivalents (with two chiasmata). It appeared that the average number of chiasmata per cell category was constant (Table I). This has also been observed by Prakken[23] and by the present author in other hybrids (unpublished). The average number of chiasmata per bivalent for all cells was 1.59. This indicates that autosyndesis of the 14 *H. jubatum* chromosomes was thus fairly strong, although not complete.

Occasionally a trivalent was formed by the *H. jubatum* chromosomes (Table I). One cell showed 22 chromosomes; the extra chromosome probably gave rise to the trivalent in that cell. Sometimes rye chromosomes formed a long loose bivalent. Such a bivalent was seen in five of the 167 cells recorded. It is noteworthy that this is a frequency of one rye bivalent per 33 cells, almost the same freqeuncy as Nordenskiöld[19] found in haploid rye.

The hybrid of the cross *H. jubatum* × *S. cereale* var. Sangaste (No. 134) showed a different behavior of the haploid set of rye chromosomes. These chromosomes frequently formed bivalents and trivalents among themselves. Occasionally a bivalent between a *H. jubatum* and a *S. cereale* chromosome was found (Figure 2). Both rye and barley chromosomes appeared to be long and stretched. This feature made the cells very difficult to analyze. Table II shows the analysis of 10

METAPHASE I OF A CELL OF *HORDEUM JUBATUM* × *SECALE CEREALE* VAR. SANGASTE
Figure 2

Photomicrograph and photodrawing of metaphase I (rye chromosomes black): six *Hordeum* bivalents, one *Hordeum-Secale* bivalent, one *Secale* trivalent with a *Secale* chromosome loosely attached; one *Hordeum* and two *Secale* univalents.

TABLE I. Chromosome associations at metaphase I in the hybrid of *Hordeum jubatum* and *Secale cereale* var. Antelope (No. 33)

Chromosome Associations				Frequencies		Average No. of		
I	II		III	No. of cells	%	rod biv.	ring biv.	Xta per bivalent
	H*	S*						
7	7			40	24.0	2.59	4.41	1.63
9	6			78	46.7	2.49	3.51	1.59
11	5			30	18.0	2.17	2.83	1.57
13	4			9	5.4	1.57	2.43	1.61
15	3			1	0.6			
10	4		1	3	1.8			
13	3		1 (22 chrom.)	1	0.6			
5	7	1		1	0.6			
7	6	1		1	0.6			
9	5	1		1	0.6			
11	4	1		2	1.2			
			Total:	167		Average: 2.40	3.53	1.59

* H—*H. jubatum* chromosomes; S—*S. cereale* chromosomes.

cells which could be definitely determined. From this it is seen that the *H. jubatum* chromosomes showed the same tendency to autosyndesis as they did in the other hybrid. By contrast, the behavior of the rye chromosomes differed by their frequent formation of associations, which is similar to that observed and described by Levan[16] in two of three haploid rye plants. Hence, the presence of the *Hordeum* chromosomes apparently had no effect on the meiotic behavior of the haploid set of rye chromosomes in the hybrid.

Anaphase I was irregular in all hybrids. Bridges, lagging and splitting univalents (mostly rye univalents) were frequently observed. Often seven *H. jubatum* chromosomes with zero to seven rye chromosomes were observed at the poles.

At interphase the diad cells often contained several micronuclei caused by the failure of the lagging chromosomes to reach the poles.

From interphase onward, anthers of most florets showed progressive degeneration. This appeared to be so for both hybrids. In this process the dividing cells died before the tetrad stage was reached. These degenerating cells could be readily distinguished by their irregular outline and rather thick appearance of the cell wall.

Most cells undergoing the second meiotic division were unable to form a spindle, probably as a result of the degeneration process. The chromosomes, scattered throughout the cytoplasm, were split into their chromatids and since the spindle mechanism failed they did not move toward the opposite poles but stayed close together. The *S. cereale* chromosomes, however, were still distinguishable. Some chromosomes did not split; these were mainly rye chromosomes which were univalents at metaphase I and had lagged and split at anaphase I (Figure 1*D*). It is remarkable that nucleoli were visible in all these cells. This indicates that the actual stage was probably prometaphase II. Studying these cells was relatively easy since the chromosomes were very short and condensed.

It was noticed that several of these cells did not appear in the diad condition but were single and rounded (Figure 1*D*). They originated from diad cells which separated and rounded off (Figure 1*C*).

As stated earlier, failure to form a spindle at metaphase II prevented the formation of normal nuclei at the poles. However, some grouping and nuclear formation still occurred. Chromosomes lying closely together formed a nucleus excluding remotely scattered chromosomes (Figure 1*E*). In this way up to six nuclei per cell were observed. Many degenerating cells, however, did not form such nuclei and the chromosomes remained visible until the whole contents had disappeared (Figure 1*F*).

Cells at anaphase II, telophase II and tetrad stages were rarely encountered (Figure 1*G*). Irregularities such as bridges, lagging chromosomes and micronuclei were common in these few cells. The fact that these late meiotic stages did occur shows that the pollen-mother-cells were intrinsically able to go through the entire meiotic cycle and to form tetrads. The degenerating process depended apparently on the physiological state of the anthers. When the dividing cells were able to go through the whole of meiosis all cells of that particular anther were able to do so. When the cells collapsed before having reached the metaphase II, all cells of that anther degenerated. It is probable, therefore, that the early degeneration process of the cells in meiotic division (usually starting after interphase) and the inability of the cells to form a spindle at metaphase II is of a physiological and nutritional nature, quite likely due to the failure of proper functioning of the tapetum cells. Degenerated and collapsed tapetum cells were indeed observed in the anthers concerned. Nordenskiöld[20] and Crowder[8] observed in triploids of *Phleum* and in *Festuca-Lolium* hybrids, respectively, the same phenomenon. Crowder[8] also observed abnormal tapetum cells.

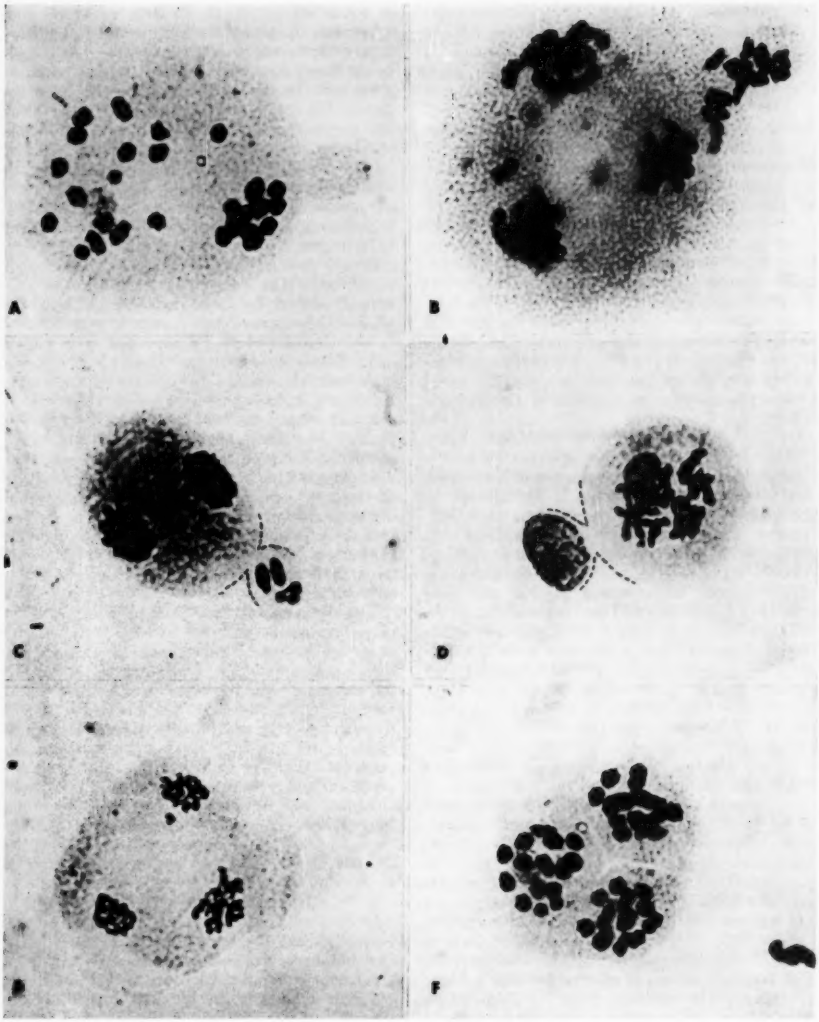
The collapse of the pollen-mother-cells before completion of their development would lead to pollen sterility, and this was confirmed when the pollen was examined. The egg cells were also completely nonfunctional, a fact understandable in view of the observed irregular chromosome behavior at pollen-meiosis and the probable structural dissimilarities existing between the *H. jubatum* chromosomes.

In a few anthers, some giant cells with supernumerary chromosomes were observed. Similar cells, probably having arisen by cell fusion (syncytes) have been previously described by several authors[8, 11, 15, 18]. In this material, the giant cells were two to six times the diameter of ordinary cells. Stages from diakinesis to telophase I were observed. Several of these large syncytes showed a tube-like protrusion with (Figure 3*B*) or without chromosomes (Figure 3*A*). It appeared that a type of 'budding off' followed the protrusion formation, giving rise to cells with a small cell connected to it again with (Figure 3*C*) or with-

TABLE II. Chromosome associations at metaphase I of meiosis in the hybrid of *Hordeum jubatum* and *Secale cereale* var. Sangaste (No. 134)

I		II			III	
H*	S*	H/H	H/S	S/S	S/S/S	No. of cells
	5	7	—	1		3
2	5	6	—	1		4
1	3	6	1	—	1	1
3	4	5	1	1		1
8	2	3	—	1	1	1
					Total:	10

*H—*H. jubatum* chromosomes; S—*S. cereale* chromosomes.



UNUSUAL IRREGULARITIES AT MEIOSIS OF *HORDEUM JUBATUM* × *SECALE CEREALE* VAR. ANTELOPE

Figure 3

A—Cell with tube-like protrusion. *B*—Cell with protrusion containing chromosomes. *C*—Tube 'budded off' into small cell with four chromosomes. *D*—'Budded off' cell with no chromosomes. *E*—Anaphase I cell with group of chromosomes located outside spindle mechanism. *F*—Late telophase I : triad.

out chromosomes (Figure 3*D*). It was also noticed that these large cells sometimes formed three groups of chromosomes in anaphase I. This was probably not due to a tripolar spindle, since it was observed that sometimes several of the supernumerary chromosomes fell outside the influence of spindle action, forming a separate nucleus (Figure 3*E*). This type of chromosome distribution probably gave rise to a three-celled association or triad at interphase (Figure 3*F*).

In conclusion it can be said that all these unusual phenomena described above resulted from the abnormal physiologic condition in the anther tissues. Haploids, triploids, etc., often show similar phenomena[8, 11, 14, 15, 20]. The triploidy of this hybrid, together with the fact that haploid chromosome sets of two species belonging to different genera have been brought together is probably the basic cause of the upset of the physiologic mechanisms of the anthers.

Discussion

The striking feature of the cytology of the hybrids under discussion was the difference in size between the rye and the *H. jubatum* chromosomes. This feature made it possible to determine the origin of the chromosomes that participated in the observed bivalent formation. Almost all bivalents proved to be *Hordeum* chromosomes; chromosome pairing was thus mainly autosyndetic. This suggests that *H. jubatum* has two closely related genomes which in the haploid condition are able to form bivalents, in spite of structural dissimilarities that probably exist between their chromosomes. Fairly strong pairing was indicated by an average of 5.9 bivalents per cell of which 3.5 (or 59 percent) were closed.

In the artificial hybrid between *Agropyron trachycaulum* ($2n = 28$) and *H. jubatum* ($2n = 28$) Boyle and Holmgren[6] observed an average of 6.3 bivalents per cell. There were apparently no differences in size between the chromosomes belonging to the two species, thus making it difficult to determine whether the pairing was auto- or allosyndetic. Although they did not rule out the possibility of autosyndesis, on the basis of the absence of quadrivalents in both *H. jubatum* and *A. trachycaulum* and the low number of multivalents in the induced octoploid[1], the authors assumed allosyndesis between an *Agropyron* and a *Hordeum* genome. Each species would then have one genome in common.

Lack of multivalent formation in parental species should, however, be regarded with extreme caution as a criterion for deciding between allo- or autosyndesis, particularly in the absence of supporting evidence. This can be explained as follows: firstly, segmental allotetraploids originating from closely related species with genome homology may show only bivalents at metaphase I. Stebbins[27, 28], and Freter and Brown[10] pointed out that selection pressure on the "raw" segmental allotetraploid in favor of increased fertility may increase quadrivalent, or, by further increase of structural differences, bivalent formation. Although in the latter case two sets of genomes homologous for significant portions of the chromosomes exist together, preferential pairing of the chromosomes in the segmental allotetraploid will result in formation of bivalents only. In the polyhaploid condition, however, such plants would show autosyndesis, since each chromosome will have a partner sufficiently homologous to pair with and to form a bivalent (Darlington's differential affinity[9]). Secondly, although multivalent formation is a usual phenomenon in true autopolyploids, this is not necessarily always the case. For example, Nordenskiöld[22, 20] reported only bivalents were formed in the autopolyploid *Phleum pratense* ($2n = 42$). Bivalents were also observed in the haploid of this species[15, 20]. This chromosome behavior is in agreement with that which has been found in haploids of such autopolyploids[16, 21]. Autosyndesis at meiosis of polyploids of both types of species, as just discussed, may also occur in hybrids involving one of those types, providing that the presence of the chromosomes of the other species in the hybrid does not genetically interfere with bivalent formation. Although autosyndesis may occur in such hybrids, the parental species exhibit only bivalents.

The observed potentiality of the *H. jubatum* chromosomes to pair autosyndetically, as was observed in the *H. jubatum* × *S. cereale* hybrid, may explain the bivalent formation in the hybrid of *A. trachycaulum* × *H. jubatum* of Boyle and Holmgren[6]. The bivalents in this hybrid are thus most likely formed by autosyndesis of the two similar genomes of *H. jubatum*. Bivalent formation has also been observed in other hybrids involving *H. jubatum*. Bowden[5] examined cytologically the artificial hybrid between *Elymus virginicus* L. ($2n = 28$) and *Hordeum jubatum* and also their natural hybrid × *Elymordeum montanense*. The first hybrid showed an average of 4.4 bivalents (in 20 cells) and the latter 6.6 bivalents (in 10 cells). The discrepancy in bivalent formation between these two hybrids may be due to the low number of cells studied, to environmental conditions[5] or to differences in strain of the parental species. The bivalent formation observed in these hybrids is likely also a result of autosynapsis of *H. jubatum* chromosomes.

The proportion of rod bivalents in the *A. trachycaulum* × *H. jubatum* hybrid of Boyle and Holmgren[6] was considerably higher than in the *H. jubatum* × *S. cereale* hybrids of the present author. This may have been due to differences of environmental conditions in which the respective hybrids were grown. However, the possibility cannot be ruled out that genes or unfavorable gene combinations were present in their hybrid that restricted somewhat the formation of chiasmata after synapsis of the homeologous chromosomes, induc-

ing more rod bivalents at metaphase I.

Since autosyndesis was fairly strong in the present hybrid and no quadrivalents were formed in *H. jubatum*, preferential pairing apparently takes place at meiosis of this tetraploid species. From these observations, together with the fact that *H. jubatum* is a self-fertilizing species[28], it may be concluded that this species is most likely a segmental allotetraploid, having arisen by natural hybridization of two closely related diploid species and subsequent chromosome doubling.

Summary

Two hybrids of the cross *H. jubatum* ($2n = 28$) × *S. cereale* ($2n = 14$), one involving var. Antelope and the other var. Sangaste as the rye parent, were reared by embryo culture on an artificial nutrient medium and transplanted to soil. Both hybrids were perennial and had a somatic number of 21 chromosomes. At meiosis the rye chromosomes could be distinguished as being larger than the *H. jubatum* chromosomes. It was thus possible to observe that the *H. jubatum* chromosomes showed fairly strong autosyndesis (average of 5.9 bivalents and 9.1 univalents). The *S. cereale* chromosomes were mostly observed as univalents. However, autosyndetic bivalent and multivalent associations among the rye chromosomes and some associations with *H. jubatum* chromosomes were regularly seen in the hybrid having the variety Sangaste as the rye parent. The fairly strong autosyndesis displayed by the *H. jubatum* chromosomes is due to two closely related genomes possessed by the tetraploid *H. jubatum*. It is probable, therefore, that *H. jubatum* is a segmental allotetraploid.

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ALBINO FROGS

THREE albino frogs live the pampered, protected life of Riley in a bathtub in the office of Florida State University zoologist Dr. John A. Davison.

Dr. Davison hopes the extremely rare frogs can be grown to maturity and that he can use them to initiate what he thinks will be the first pure strain of albino frogs in the world.

The albinos showed up as an accident of nature in some cross-breeding of two completely normal-colored grass frogs Dr. Davison crossed to get tadpoles for research on blood circulation.

Through a slip-up in nature the fragile-looking white frogs are completely lacking in pigment, the material that gives color to the skin. Some of the five normal-colored frogs produced in the crossing also carry the gene to produce albino offspring.

Albinism, carried by a recessive hereditary gene, occasionally crops up in rats, salamanders and higher animals including man. It is considered extremely rare in frogs.

So far as the scientist has been able to determine, he has the only albino frogs in this country. "The only albino frog in any other country about which I have heard is an adult male discovered in nature recently in England," Dr. Davison said.

Dr. Davison says he won't be able to determine the sex of the three albinos until they are about half-grown, in at least another four months. But if there isn't a male and a female in this group he thinks there is sure to be in the entire family of eight frogs.

Some of the five normal-colored frogs also carry the recessive albino gene and can be used to breed albino offspring. If the albinos are all one sex, he will cross them with the normal-colored frogs of opposite sex from the same family to get a nursery full of little frogs one-half of which will be normal-colored and one-half albino.

After producing a pure strain, Dr. Davison plans to give the offspring to other scientists who are conducting research in genetics, pigmentation and embryology.

The scientists will have to wait for their research animals. It takes a year or more to raise frogs to the breeding stage.

In the meantime the albinos and their five normal-pigmented brothers and sisters are living it up in a bathtub bungalow complete with a rock patio for lounging and private swimming pool. Their diet of choice mealworms is fed them by the scientist himself.

The normal frogs accept their three albino brothers and sisters as full members of the family despite their off-beat appearance. The darker frogs don't appear to look down upon the white frogs in the least, he said.

As for the white ones Dr. Davison said that despite some theories that albinos don't see well because of lack of pigment in the iris of the eye they appear to see all right and compete very satisfactorily for their share of the live mealworms he feeds them.

In nature, he said, they wouldn't fare so well. In the natural environment their lack of protective coloring, which would make them indistinguishable from a leaf or a twig, would make them fall easy prey to birds, snakes and other predators out for a good plate of frog-legs. This may account for the fact that they are so rarely found in nature.

LIFE SPAN IN INBRED AND HYBRID MICE

C. K. Chai*

THE life span of individuals in a population is an important component of fitness, which hybridization is known to confer. It may be expected, therefore, that heterozygosity is correlated with life span. The aim of this study is to determine the effect of heterozygosity on life span in individuals of a population as measured by a number of parameters, including means, distributions and maximum life span. Specifically, the experiments were designed to find out how hybrid populations differ from their parental inbred populations in these parameters. Although the correlation of heterozygosity with life span may be different from one species to another, answers concerning these questions may contribute to the understanding of the genetic aspects of ageing. It has been demonstrated that F_1 hybrids live longer than their parental strains as measured by both mean and maximum life[3] in *Drosophilia*. Data are lacking concerning life span in genetically different populations of mammals. Since mammals have been widely used in the study of ageing, data on life span in hybrid and inbred mice, aside from genetic significance, should serve as background information for studies on the process of ageing.

A study of lifespan of inbred and hybrid mice is given in the present report. This has led to an interpretation of the effect of hybridization and genetic constitution on life span.

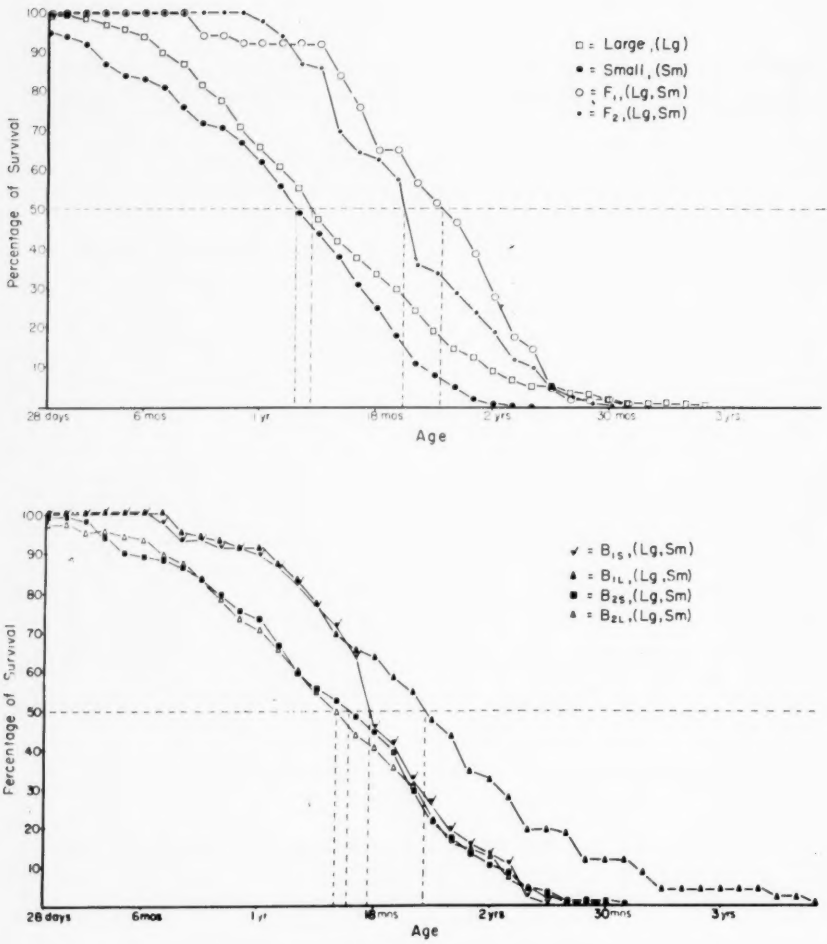
Materials and Methods

The mice used in this study were the Large and Small strains and crossbred generations of Large with Small, DBA/1 and BALB/c. The Large and Small mice were developed by selection for many generations for large and small body sizes respectively. Selection was followed by inbreeding by means of brother-sister mating. The detailed history has been reported elsewhere[1]. The mice included in this study were from the third to ninth generations of sib-mating for the Large and 14th to 22nd for the Small strain. Strains DBA/1 and BALB/c are well established inbred strains. The development and characteristics of them need not be described here.

The following list gives the crossbred types being used in this study and the designations for each.

Parental strains:	Large (LG), Small (SM)
Hybrids:	F_1, (LG, SM)
	F_2, (LG, SM)
	1st backcross to LG, B_{1L}, (LG, SM)
	1st backcross to SM, B_{1S}, (LG, SM)
	2nd backcross to LG, B_{2L}, (LG, SM)
	2nd backcross to SM, B_{2S}, (LG, SM)
Parental strains:	LG, DBA/1
Hybrids:	F_1, (LG, DBA/1)
	F_2, (LG, DBA/1)
	1st backcross to LG, B_{1L}, (LG, DBA/1)
	1st backcross to DBA/1, B_{1D}, (LG, DBA/1)
Parental strains:	LG, BALB/c
Hybrids:	F_1, (LG, BALB/c)
	F_2, (LG, BALB/c)

* Roscoe B. Jackson Memorial Labratory, Bar Harbor, Maine. This investigation is supported by grant C-3857 and RG-5550 from the National Cancer Institute of the National Institutes of Health, Public Health Service. The author is grateful to Mrs. Marion Dunbar and to Rosalie Gwynn for their assistance in collecting the data.



SURVIVAL OF LARGE AND SMALL STRAINS

Figure 4

Plots of percentage of survival against age from 28 days (weaning) to three years or more on a month interval for mice in the Large and Small strains and their F_1 and F_2 crosses (above) and the first and second backcrosses (below). The median for each group was extrapolated.

The crossbred generations were produced by reciprocal crossing with respect to the parental types, except B_{1L}, (LG, DBA/1) and B_{1D}, (LG, DBA/1), which were produced by matings of F_1, ♀ × LG, ♂ and DBA/1, ♀ × F_1, ♂ respectively. Most of the animals have been used for studying quantitative inheritance of body size[1,2]. The data for the life span of the parental strains of DBA/1 and BALB/c were incomplete and excluded from the analysis. For this reason the data for the LG, SM and their crosses constitute the main body of this study and data for the additional inbreds and hybrids substantiate observations on LG, SM and crosses between them.

All mice included in this study were born between September 1952 and September 1956, and included males, virgin females and a few females that had been used as breeders. Occasional animals that escaped or were accidentally lost were excluded from the data. Only animals that were observed until death are included in the analysis of the data, except four mice in the B_{2L}, (LG, SM) group which are still alive. All the mice were fed with Purina Laboratory Chow and given water *ad libitum*. They were housed in an animal room maintained at a temperature of about 75°F. All were weaned at 28 days of age. Males and females, except breeders, were kept separately with a maximum of five in a pen. Pens were checked weekly for dead animals. Preweaning deaths were not included in the data.

Results

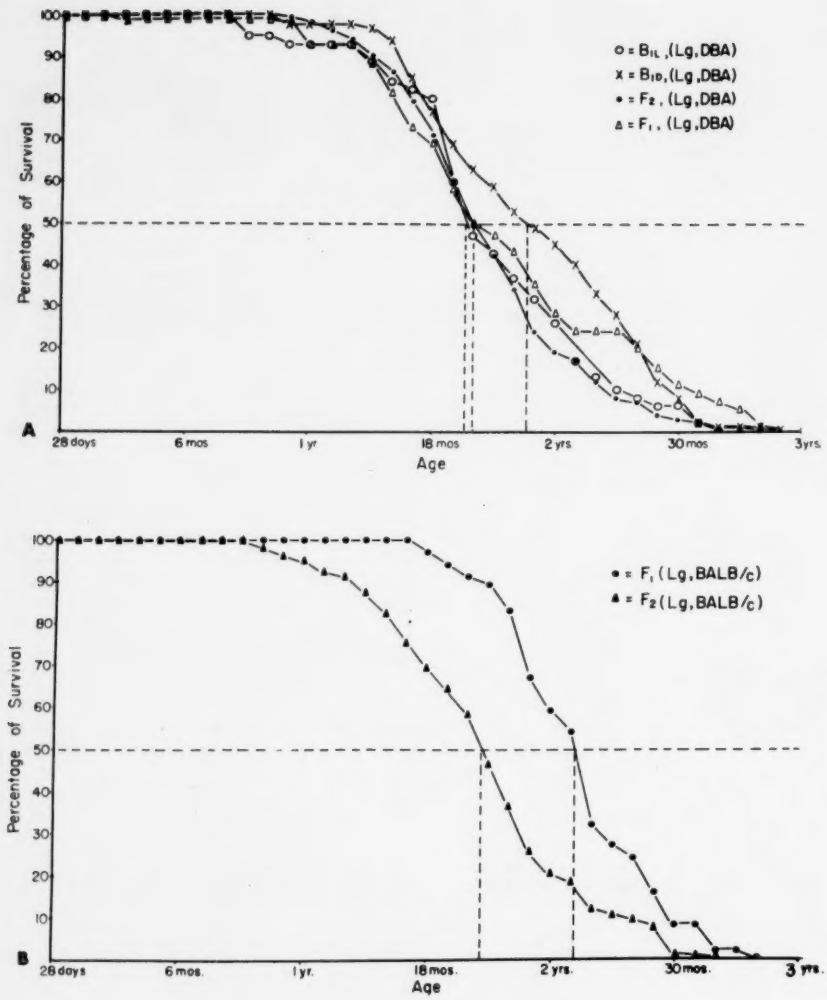
The mean, standard deviation, median and the difference between mean and median of life span were calculated for each genetic group (Table I). The percentage of survival from weaning (28 days) up to 3½ years in each group was plotted on a month interval basis (Figures 4 and 5). For illustration, the median in each group was estimated graphically.

In the groups of LG, SM and their generations, the F_1 and B_{1L} had longest mean life spans, 644 and 643 days respectively. The means of the backcrosses to either parental strain decreased in two successive backcross generations. The difference between the mean and median was greatest in the LG, indicating a skewed distribution to the right with more old animals in this group. The similar means and medians in each of the groups of SM, B_{2S} and F_1 indicate symmetrical distributions, but the coefficient of variation was greater in the SM and B_{2S} than in F_1. This difference in variation is more easily visualized by examining the distribution curves (Figure 4).

It is of interest to see that, together with large mean and standard deviations, the B_{1L} (LG, SM) has a survival curve with the longest stretch toward the right. This indicates a relatively large propor-

TABLE I. Means and medians of age at death in days in inbred and hybrid groups of mice

Genotypic Groups	Number of Mice	Mean	Standard Deviation	Coef. of Variation	Median	Difference Mean - Median
LG	227	491	200	40.7	443	48
SM	171	422	180	42.7	416	6
F_1(LG,SM)	38	644	190	29.5	645	−1
F_2(LG,SM)	58	607	150	24.7	582	25
B_{1L}(LG,SM)	77	643	220	34.2	618	25
B_{1S}(LG,SM)	46	568	140	24.6	529	39
B_{2L}(LG,SM)	201	492	200	40.7	476	16
B_{2S}(LG,SM)	156	496	210	42.3	500	−4
F_1(LG,BALB/c)	107	756	140	18.5	755	1
F_2(LG,BALB/c)	122	645	150	23.3	621	24
F_1(LG,DBA/1)	53	644	170	26.4	607	37
F_2(LG,DBA/1)	284	645	140	21.7	603	42
B_{1L}(LG,DBA/1)	46	650	150	23.1	595	55
B_{1D}(LG,DBA/1)	71	711	150	21.1	685	26



SURVIVAL OF LARGE, DBA/1 AND BALB/c MICE

Figure 5

Plots of percentage of survival against age from 28 days (weaning) to three years or more on a month interval for mice in crosses between Large and DBA/1 (*A*) and between Large and BALB/c, (*B*). The median for each group was extrapolated.

tion of older animals. Indeed, the survival curve shows that a few animals had lived more than three years of age, an age which is extremely rare in domestic mice (Medawar[4]).

Three of the four crossbred groups from LG and DBA/1 are rather similar in life span based on all statistical parameters used, the fourth, B_{1D}, showed somewhat larger mean values and their percentage-survival curve lies slightly to the right of the others. Of the two hybrid groups of the LG and BALB/c cross, the F_1's were longer lived than the F_2's according to the means and medians. From the distribution curve it may be seen that a great part of the difference is caused by difference in mortality before the age of 1½ years. All the mean and medians of these hybrid groups are greater than that of the parental LG strain, but there were Large mice which had lived as long as the oldest of these hybrids.

Since the differences in the pattern of distribution of life span exist in the different groups and since the genetic meaning of the differences is not understood, an attempt to adjust the data for test of significance between the groups has not been made. Nevertheless, the means and medians of all three F_1, F_2 and four out of six backcross groups were larger than either of the LG or SM parental strains.

Discussion

Hybridization had an effect on life span in mice as revealed by the means and the curves of percentages of survival in the inbred strains and hybrids. Although some of the differences may not be statistically significant, the means of the F_1 and F_2 hybrids were larger than those of the parental strains. The major contribution to the differences was differential mortality at earlier ages where the death rate of inbreds was higher than that of the hybrids. Indeed, in the F_1 groups practically none died before reaching one year of age, whereas the inbreds started to die from the weaning age. (Death before weaning was not considered). Data on *Drosophila*[3,7] showed similar tendencies, although most of the F_1's also lived longer than their parents.

Differential mortality between inbreds and hybrids requires genetic explanation. Segregation of lethal and semi-lethal genes and decline of homeostasis with decrease of heterozygosity are main causes of inbreeding degeneration. The importance of these properties contributing to the earlier deaths in the LG and SM mice is hard to evaluate. In view of the consistency of their body sizes through the many generations of inbreeding in addition to the long history of selection, we may judge that genes determining body size have been fairly well fixed in these two strains. This selection for body size and inbreeding does not suggest, however, that genetic units determining longevity have also reached a high degree of homozygosity, in view of the fact that natural selection is generally in favor of heterozygotes. Furthermore it is not known whether or not size genes have pleiotropic effects on longevity. It is rather certain however that the genetic consequences resulting from the breeding scheme have caused reduction of fitness accompanied by a shift of body size to the extremes found within populations of mice.

Longevity may be treated as a metrical character of continuous variation. With the exception of lethal and semi-lethal factors, polygenes may be considered as the main genetic units determining the variation. Organisms of certain genotypes, depending on the action of the individual genes and interaction between them, enjoy high fitness and are long lived. In other words, an organism with a "balanced genotype" so to speak, may postpone its senescence. This phenomenon has not been overshadowed by heterosis, in view of the fact that some of the crosses, such as B_{1L}, (LG, SM), had more animals with extremely long life—longer than any of the other hybrids.

In summary, the present experimental results apparently favor the following conclusions: Superior physiological versatility in the hybrids leads to the reduction of early mortality but not to postponement of senescence. Genetic constitution, of the individual, or concerted effect of genes, determines the onset and advance of senescence. Individuals endowed by heterozygosity with versatile physiologic characteristics, so called self-regulation or buffering, are better able to cope with varying environment. This property is more critical during the period of development and growth than in later life. The validity of these conclusions needs proof from more experimental evidence.

The Large mice were susceptible to skin infection by fungi or external parasites. Deaths sometimes occurred from malnutrition, secondary to the infection. Lymphomas have been observed in these mice at old ages. The Small mice are leukopenic and old mice have been found with kidney infections. Mammary tumors were observed in the crosses between the LG and DBA strains. Causes of death due to or secondary to these pathologic conditions have not been reported here. They undoubtedly cause reduction of mean life span. The relationship of causation and effect in some of these conditions with ageing is not understood at present. In any event, if we care to accept the

fact that these conditions are strain characteristics and genetically determined, inclusions of death directly or indirectly due to these causes in the estimation of the life span, may not be considered as bias from the genetic standpoint. We have not calculated the life spans separately for the two different sexes in each group. Although most of the observed pathologic changes, except mammary tumors, are unlikely to occur more in one sex than in the other, sex differences in life span among the different genetic groups could not be wholly eliminated.

The life tables for inbred strains of mice differ greatly[5,6]. Genetic differences in ageing, susceptibilities to infections and neoplasia as well as environmental differences may all contribute to life span variation between groups. In addition, differences in methods of collecting data also contribute to strain differences in life span. For example, some investigators included in their data only animals which had reached two or more months of age. Those which had died before reaching this age were disregarded. Others included only animals dying within a certain time interval, while animals in the same population born contemporarily were still alive. The former procedure of collecting data would result in overestimation and the latter in underestimation of the mean age for animals in the studied populations as compared with the results presently obtained. Because of the differences in procedures of collecting life span data, no valid comparison of our data may be made with those published by others. As far as the completeness of the data is concerned, it would be desirable to have life tables for the DBA/1 and BALB/c strains. The data presented here are unique in the sense that all animals were born during a given time interval and all but four were observed throughout their life span.

Summary

Life tables are given of inbred mice of the Large and Small strains and their hybrids, F_1, F_2, 1st and 2nd backcrosses in addition to mice of the crossbred generations F_1, F_2 and 1st backcrosses between Large and DBA/1 and F_1 and F_2 between Large and BALB/c. The Large mice had longer mean life than the Small mice. In general, the hybrids had longer mean life and lower mortality at earlier ages than that of the parental inbred strains. Older animals with extremely long life were observed in the group of 1st backcross to Large. It has been speculated, based on these results, that both hybridization and genetic constitution affect life span in individuals of a population. The former tends to permit development and growth and reduces early mortality and the latter determines the onset of senescence.

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SEX-LINKED DWARFISM IN THE FOWL

F. B. HUTT*

SOME years ago Mr. E. N. Larrabee of Peterborough, New Hampshire, kindly provided for study three "short-legged" pullets which were said to be typical representatives of some peculiar dwarfs that had appeared from matings of his pure-bred New Hampshires of normal size. The average weight of these three females at maturity was 1727 grams, which is about 800 grams below average weight of adult, normal hens of that breed.

Subsequent studies showed that this remarkable reduction in size was caused by a single sex-linked gene. Brief summaries of its effects were given earlier[4,5], and details are reported herewith.

Inheritance

When mated with a Barred Plymouth Rock male, and (later) another large male, the three dwarfs produced in the F_1 generation only offspring of normal size—no dwarfs. In later years, subsequent matings showed clearly that the dwarfism is caused by a sex-linked gene which is apparently completely recessive in heterozygous males. The symbol *dw* was assigned[4]. Typical results from matings of four different kinds, in which 14 sires yielded 2,307 classified offspring, are given in Table I.

Unlike some other kinds of dwarfed or stunted chickens, these sex-linked dwarfs show no conspicuously disproportionate development of any part of the body and no bulging of the cranium. For that reason, and because the ranges of size in dwarfs and full-sized birds overlap for more than three months after hatching, classification is difficult at earlier ages. Growth curves for groups show significant retardation in the dwarfs by six weeks of age, but individual birds can sometimes not be classified with certainty until 10 to 15 weeks of age, or even later.

Effects on Growth

A. *Of Embryos*

To determine whether or not the gene *dw* affects growth of the embryo and chick during the three weeks of incuba-

TABLE I. Matings showing sex-linked transmission of the gene for dwarfism, *dw*

Sires			Progeny			
			Males		Females	
Number	Genotype	Dams	Normal size	Dwarf	Normal size	Dwarf
6	*Dw dw*	Normal size	707	0	340	347
3	*Dw dw*	Dwarf	126	103	118	111
3	*dw dw*	Normal size	147	0	0	127
2	*dw dw*	Dwarf	0	82	0	99

* Department of Poultry Husbandry, New York State College of Agriculture, Cornell University, Ithaca, New York. Number 27 in the series by F. B. Hutt entitled "Genetics of the Fowl." The author takes pleasure in thanking Mr. Robert Marshall of Ithaca, for rounding up and contributing dwarf Leghorns, also Dr. C. D. Mueller, who, when a graduate student here, took the weights shown graphically in Figure 6 and helped in other ways, and Mr. Keith Thompson of the Biometrics Unit, Department of Plant Breeding here, for statistical analyses and straight lines that saved the author from an erroneous interpretation. Finally, to that knowledgeable poultry breeder, Mr. E. N. Larrabee of Peterborough, New Hampshire, special thanks for recognizing that his little hens could brighten the life of some geneticist, and for selecting this one for that privilege.

tion, 402 chicks, all hatched on April 18, 1944, were weighed individually at hatching. These all came from comparable matings that would yield *Dw* − and *dw* − full sisters in approximately equal numbers. Furthermore, they were all hatched from hens not dwarfed that laid eggs of normal size, about 57 to 60 grams. Such eggs could impose no limitation on the initial size of the chick, as would the smaller eggs laid by dwarf hens.

Later on, when the birds had been raised and classified for size and sex, mean weights of the females at hatching were found to have been almost identical (Table II). It seems clear, therefore, that the gene *dw* does not retard growth of the chick during incubation.

B. *After Hatching*

Effects of that gene on growth after hatching were determined by weighing individually some or all of about 300 birds every four weeks from two to 26 weeks of age. These were hatched at two-week intervals from March 18 to May 6, 1942, and (after the usual period in brooders) were reared on an open grass range. Because of predators, mortality and gallinaceous wanderlust, some birds were missed at one or more weighings, but complete records (seven weights) were obtained for 207 birds. These came from three different matings as follows:

A-1. ♂ J 4103 *Dw dw* × 6 ♀♀ *dw* −, yielding dwarfs and normal birds of both sexes from eggs with average size (per dam) of 42.1 grams; 38 birds with complete records.

A-2. ♂ J 4103, as above, × 3 ♀♀ *Dw* −, yielding normal males, normal females and dwarf females in the ratio of 2:1:1 from eggs that averaged 54.7 grams in weight; 38 birds.

B. As A-2, but with a different sire and different dams. Their eggs had an average weight of 60.1 grams; 131 birds.

The resultant data (Figure 6) show that growth of the dwarfs, both male and female, was consistently retarded by six weeks of age and continued thereafter at a slower rate than growth of normal siblings. It seems clear that the effect of the gene is not to stop growth at any specific age, but rather to retard that process during the whole of the normal period of growth. This is quite different from the action of the gene causing pituitary dwarfism in the mouse, which induces an almost complete cessation of growth at 17 to 35 days of age[1]. Actually the retarding effect of *dw* in females is evident (but not in Figure 6) by two weeks of age. Mean weights at that age of full sisters from Mating B (large eggs) as given in Table II show that the dwarfs were then significantly smaller than their sisters that later attained full size. It is evident, therefore, that the gene *dw* begins to retard growth sometime in the first two weeks after hatching.

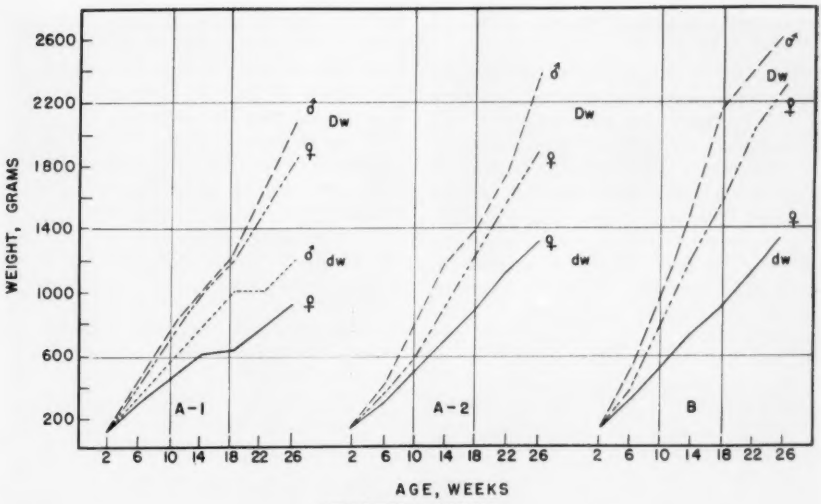
C. *Maternal Influence*

In birds from Matings A-2 and B that were not dwarfed, the usual more rapid growth of males than of females was clearly evident by six weeks of age. In contrast, among chickens not dwarfed from Mating A-1, males did not exceed females in weight until after 18 weeks of age. Failure of these birds to show the usual sex dimorphism by that time may have resulted in part from the small

TABLE II. Weights at early ages of dwarfs and their normal sisters

Genotype	At hatching		At two weeks	
	Chicks, number	Weight, grams	Chicks, number	Weight, grams
♀♀ *Dw*— (normal)	90	41.8 ± 0.38	30	116.13 ± 2.58
♀♀ *dw* — (dwarf)	94	41.9 ± 0.42	27	103.37 ± 3.22
Difference		none		12.76 ± 4.13*

*Significant



GROWTH CURVES

Figure 6

Growth curves for dwarfs and their normal siblings showing gradual retardation of growth in the former. Those hatched from small eggs (A-1) still lagged behind dwarfs from large eggs (A-2, B) at 26 weeks of age.

numbers weighed (13 ♂♂, 9 ♀♀), and possibly from effects of the gene *dw* in the males. All of these were *Dw dw*, whereas the females were all *Dw* —. However, such heterozygous males are apparently of normal size as adults when hatched from eggs of normal size. It seems more likely, therefore, that the males from A-1 were subnormal in size (up to 18 weeks at least) because of the limitation imposed on their size and growth by the small eggs from which they hatched.

As several investigators have shown, initial size of the chick is closely related to the size of the egg from which the chick hatches. The curves in Figure 6 show that, in general, comparable chicks from the small, medium and large eggs of Matings A-1, A-2 and B, respectively, still ranked in that same order by 18 weeks of age. Exceptions were the dwarf females from A-2, which grew as well as the dwarfs from B, and the non-dwarfed females of A-1, which matched the growth of their larger half-sisters from A-2. Since, under optimum conditions, retardation of growth in females is normally evident before six weeks of age, it is not surprising that the females should be less handicapped than males when both are hatched, as in A-1, from eggs disproportionately small in relation to the genotype of the chicks. Persistence of that handicap may account for the comparatively slow growth of the A-1 males to 18 weeks of age. If so, the effect is similar to the persistent maternal limitation of growth and size in foals from the cross of Shire ♂ × Shetland pony ♀♀ as described by Walton and Hammond[14].

It seems probable, however, that the maternal limitation of size in sons of dwarf hens is eventually outgrown. For eight such males the average weight at 10 to 12 months was 3243 grams, a figure not significantly different from that of 3283 grams for three proven heterozygotes from hens not dwarfed that laid large eggs.

Effects on Adult Size

A. *Of Males*

The weights of males given in Table III show that the gene *dw* reduces the size of adult homozygous males, as com-

pared with heterozygotes, by about 43 percent.

In Series 1, 2 and 3 of that table, the two groups compared are full brothers or half-brothers from a common sire, and all are from dwarfed mothers. As their ages indicate, none had reached definitive weight, but it was not practicable to keep many of them any longer. The reduction in size shown in Series 1 is unduly high, probably because of chance variation in the limited numbers. In Series 2 average age of the normal males was 13 days younger than the dwarfs and apparent reduction of the latter was only about 38 percent. In some of these same birds kept and reweighed 33 days later on November 22 (Series 3), the dwarfs were 42.3 percent smaller than the heterozygotes (see Figure 7*A*). This reduction is close to that for Series 4. The weights in that group apply to males used in different years for breeding, and were taken when the birds were put in the breeding pens in February or March.

Critical data are not available to show whether or not *dw* reduces the size of heterozygotes. To get them one would have to determine by progeny tests the genotypes of a number of the normal-sized males from matings of the type ♂ *Dw dw* × ♀ *Dw* —. Proven heterozygotes could then be compared in weight with males found not to carry the gene. Facilities for such tests were not available. However, among such males, half of which must have been heterozygous, there was no indication of a bimodal distribution with respect to size. All of them seemed equal in size to males of normal Plymouth Rocks, Rhode Island Reds and other large breeds. This is evident in the average mature weight for 11 known heterozygotes given in Table III. It seems probable, therefore, that *dw* is completely recessive.

B. *Of Females*

To determine whether or not the gene has any adverse effects on the viability and reproduction of dwarf females, 1,213 chicks were hatched in 1944 from four sires, all *Dw dw*, each penned separately with 16 *Dw* — females. Two of them (full brothers) were mated with New Hampshires, and two (from the same sire as the others) with White Leghorns.

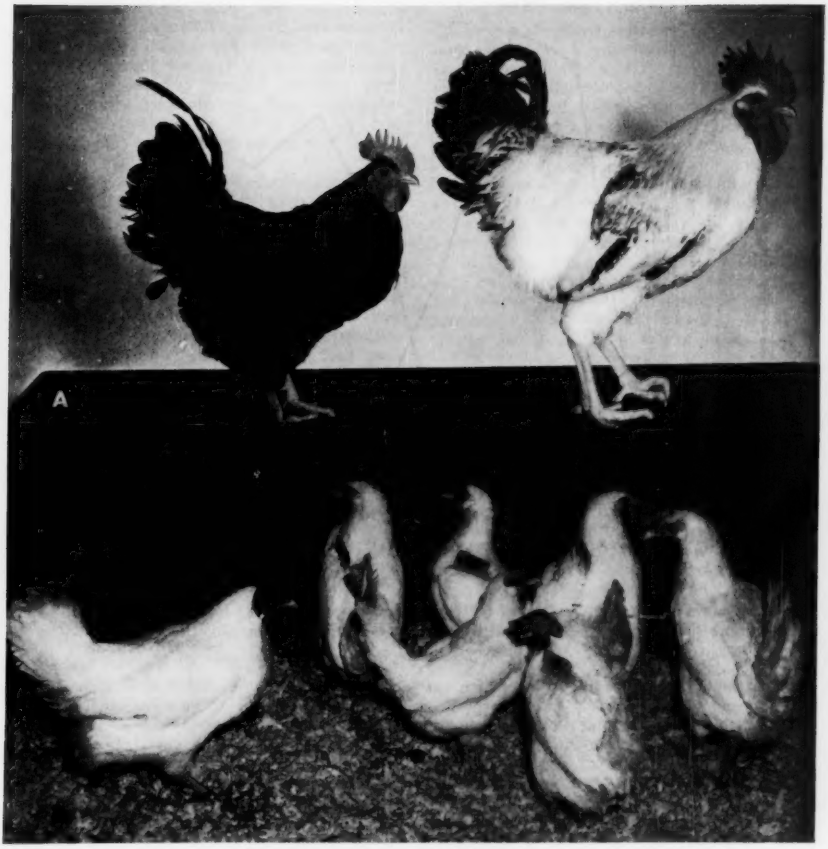
TABLE III. Weights at or near maturity of males heterozygous or homozygous for *dw*

	Dw dw			*dw dw*			
Series	Average age	Number	Average weight, grams	Average age	Number	Average weight, grams	Reduction, percent
1	162 days	6	2212	162 days	3	1197	45.9
2	170 days	13	2325	183 days	35	1458	37.7
3	199 days	8	2965	209 days	13	1712	42.3
4	10-12 months	11	3254	10-12 months	3	1748	43.2

TABLE IV. Definitive weights of dwarf females and their non-dwarfed sisters

	Dams			Daughters *Dw*—		Daughters *dw*—			
Sire's weight, grams	Breed	Ave. weight, grams	Mid-parental weight,* grams	Number	Average weight, grams	Number	Average weight, grams	Reduction in dwarfs Grams	Reduction in dwarfs Percent
♂ 6 — 3450	New Hamp.	2678	2685	77	2574	68	1748	— 826	32.1
♂ 7 — 4190	New Hamp.	2538	2903	40	2603	43	1934	— 669	25.7
♂ 8 — 3690	Wh. Leghorn	1982	2430	41	2300	40	1571	— 729	31.7
♂ 9 — 3325	Wh. Leghorn	2137	2365	62	2534	87	1817	— 717	28.3

*After reducing the sire's weight by 22 percent to convert to comparable weight for genetically similar females; the figure is calculated from the 28 percent determined by Waters[18] for the reciprocal conversion in birds of similar size.



DWARFS AND NORMAL

Figure 7

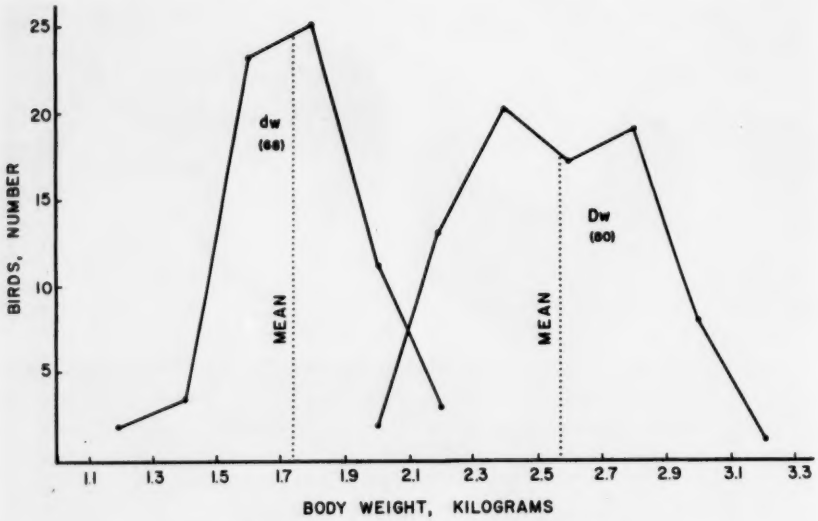
A—Full brothers, one gold, the other silver, both hatched on the same day, photographed at 32 weeks. At 30 weeks their weights were 3200 grams for the big male (*Dw dw*) and 1870 grams for his dwarfed brother (*dw dw*), a reduction of over 41 percent. *B*—Five dwarf White Leghorns flanked on either side by normal Leghorns of the strain.

Daughters from these four matings were eventually weighed in March, 1945, at which time they were 11 to 12 months old and had reached definitive size.

Comparisons of 223 non-dwarfed hens with their 236 dwarfed full sisters and half-sisters (Table IV) showed that the latter were reduced in size by 25.7 to 32.1 percent in the different matings. Within that range, the degree of reduction was unrelated to the size of (1) their normal sisters, (2) their dams, (3) their sires, and (4) the mid-parental weights. The average (unweighted) reduction in the four groups was 29.5 percent. There was very little overlapping of the range in body weights for dwarfs and for their big sisters (Figure 8).

Effect on Sexual Maturity

Age of pullets at sexual maturity is usually taken as the age at laying of the first egg. From the matings of 1944 just described, there



ADULT WEIGHT OF DWARFS AND NORMAL

Figure 8

Distributions of adult weights of 68 dwarf females and their 80 big sisters and half-sisters all from one sire, J 6. They show that there is very little overlapping of the two types.

were no fewer than 520 pullets for which age at first egg was recorded, but, in spite of those numbers, and to some extent because of them, it is difficult to state exactly what effect the gene *dw* has on sexual maturity. Of the 520 records, 265 were for dwarfs and the remainder for their big sisters and half-sisters. These came from four different sires and from three different hatches—those of March 21, April 4, and April 18. Dwarfed pullets were housed by themselves in two large pens, and their non-dwarfed sisters went in two comparable pens. No artificial lighting was supplied.

Age at first egg normally varies greatly because of genetic and environmental influences. Among the latter, one of the most important is the decreasing length of day in the autumn which reduces the stimulatory action of light and retards egg-laying in late-hatched birds. In this case, the variation among sire families and among hatches from the same sires was too great to permit lumping the data together. Accordingly comparisons of *Dw* and *dw* sisters are given separately for daughters of each sire in each hatch (Table V). In these 12 groups the numbers of females ranged from 22 to 59, the average being 43.3, and, as the marginal totals of the table show, dwarfs and big sisters were about equally represented in each hatch.

It will be noted that big daughters of ♂ J 7 matured comparatively early, while those from

TABLE V. Mean ages at first egg for *Dw* and *dw* sisters, those for the latter shown as deviations from the means for the former

Sire and dams	Hatched Mar. 21		Hatched Apr. 4		Hatched Apr. 18		Total birds, number
	Dw	*dw*	*Dw*	*dw*	*Dw*	*dw*	
♂ 6 × New Hampshires	185	− 2	179	+46	213	+16	164
♂ 7 × New Hampshires	163	+10	168	+49	184	+34	98
♂ 8 × Wh. Leghorns	174	+22	169	+58	232	+22	103
♂ 9 × Wh. Leghorns	205	+ 3	192	+26	247	+ 8	155
Total birds	73	81	98	93	84	91	520

♂ J 9 were several weeks later. Big sisters of the middle hatch which should have matured about a week later than those hatched on March 21[2], went contrary to that expectation in three of the four groups. In that same hatch, dwarfs from all four sires lagged behind their big sisters by nearly four to eight weeks, the difference being far greater than in the other hatches.

As the mean ages at first egg for the dwarfs exceeded those for their big sisters in all 12 comparisons except one, it would be easy to conclude that the gene *dw* significantly retards sexual maturity. Other considerations suggest that the dwarfs did not get a fair chance to show what they could do. It was not recognized until late in October that these birds had difficulty in feeding from ordinary hoppers because they could not easily reach the feed from the perch. Moreover, feeders designed for big birds were too high from the floor for little ones. Many of the dwarfs flew with ease to the trap-nests, but others, either less ambitious or more intelligent, preferred to lay in a dark corner on the floor. All these conditions were corrected on October 23, and the numerous "floor-layers" were eventually apprehended and taught to lay in the nests. However, as only eggs laid in the trap-nests could be credited to their producers, for a number of these birds the recorded age at first egg was much later then the true one.

It seems probable that such complications, when superimposed on the daily diminution of stimulating light in October, were responsible for the fact that mean recorded ages at first egg for the eight groups of dwarfs in the two later hatches ranged from 217 to 254 days. Corresponding figures for dwarfs of the first hatch, which were somatically mature in September, when the days were still longer than the nights, were 173 to 208 days. For 25 dwarfs of that hatch from Pen 6, the mean age was actually two days less than for their 33 big sisters Altogether it would appear that additional and better controlled data are necessary to show whether or not the gene *dw* delays sexual maturity.

Effects on Egg Production

The complications described in the foregoing section made it impracticable to measure egg production of the dwarfs (of 1944) in the usual way by the number of eggs laid from the first one to some arbitrary date or age. Instead, capacity to lay in the dwarfed hens was compared with that in their sisters of normal size by considering the numbers of eggs laid in the three months of highest production. These were March, April, and May. By that time there were few eggs not laid in trap-nests. Variation from differences in age at the onset of laying, from error in determining that age, and from partial records of hens that died, were eliminated by restricting the comparisons to birds that laid in each of the three months and lived to the end of May.

The resulting data showed that in the four sire families the average numbers of eggs laid by the dwarfs were from 7.9 to 13.3 less than the corresponding figures for their full-sized sisters from the same sires (Table VI). Expressed otherwise, the dwarfs laid from 12.7 to 18.6 percent fewer eggs than their big sisters in three months. These differences were statistically significant in every case.

It will be noted that the average rate of laying (the proportion actually laid of the 92-egg maximum that could be attained by laying an egg every day) did not exceed 63.4 percent in the dwarfs, but ranged from 67.5 to 77.6 percent in the four groups of big sisters (Table VI).

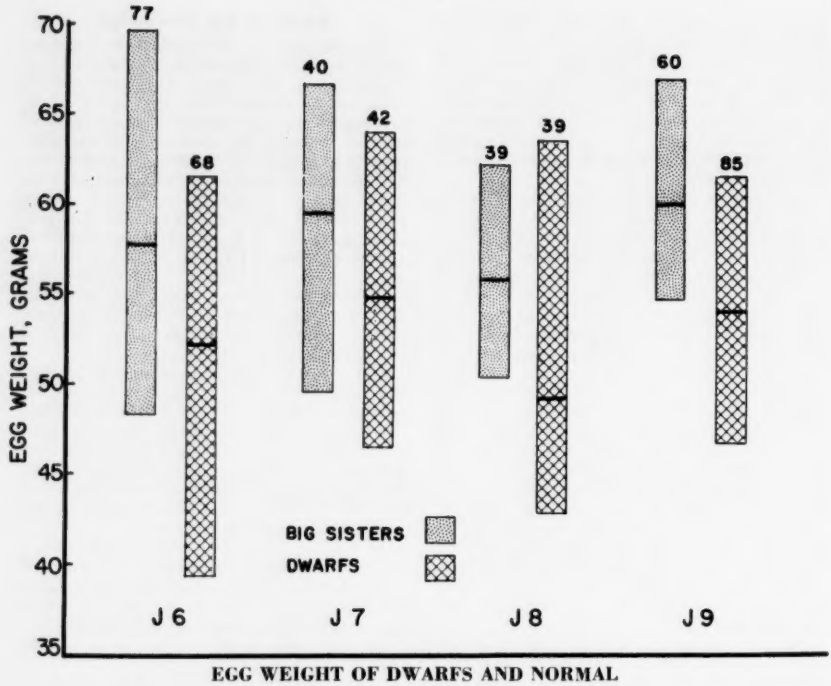
Effects on Size of Egg

Some of the dwarfs appeared to lay eggs that were unusually large in relation to body size. Definitive egg weights (averages of four to seven eggs per bird) were recorded for 238 dwarfs and their 220 big sisters that were laying in the third week of March, 1945. At that time these hens were 11 to 12 months old. In all four sire-families, mean egg weight per bird was significantly smaller for the dwarfs than for their big sisters from the same sires (Figure 9). The differences in the four families varied from 4.8 to 6.5 grams.

TABLE VI. Egg production of dwarfs and their full-sized sisters from four sires, as measured in three consecutive months

Sire and dams	Not dwarfed		Dwarfs		Diff.	Rate of laying, percent	
	Hens, number	Eggs, mean number	Hens, number	Eggs,* number	St. E.	*Dw*—	*dw*—
♂ 6 × New Hampshires	80	67.9 ± 12.8	76	— 9.6 ± 2.0	4.8	73.8	63.4
♂ 7 × New Hampshires	41	65.2 ± 17.6	51	— 9.3 ± 3.5	2.7	70.9	60.8
♂ 8 × Wh. Leghorns	40	71.4 ± 11.4	38	—13.3 ± 2.7	4.9	77.6	63.2
♂ 9 × Wh. Leghorns	61	62.1 ± 12.3	84	— 7.9 ± 2.0	3.9	67.5	58.9

*Expressed as the difference of their mean number from that for their full-sized sisters



EGG WEIGHT OF DWARFS AND NORMAL

Figure 9

Ranges in egg weight for dwarfs and their big sisters from four sires, J 6—J 9. Means, shown by the horizontal bars, were consistently lower for the dwarfs. Figures above the columns tell the number of birds in each group.

Expressed otherwise, average reduction of egg size caused by the dwarfing gene was about 10 percent, whereas average body weight of these same dwarf females was reduced by 29.5 percent. Variation in egg size was slightly but consistently greater in the dwarfs than in their big sisters. This is shown by the greater ranges (Figure 9) and higher coefficients of variability (Table VII) for the dwarfs. As average egg size in relation to body size was consistently higher for the dwarfs (Table VII), all these data tended to confirm first impressions and to show that the dwarfs lay rather big eggs in relation to their body size.

TABLE VII. Comparisons of dwarfs and big sisters in variability and proportionate weights of eggs*

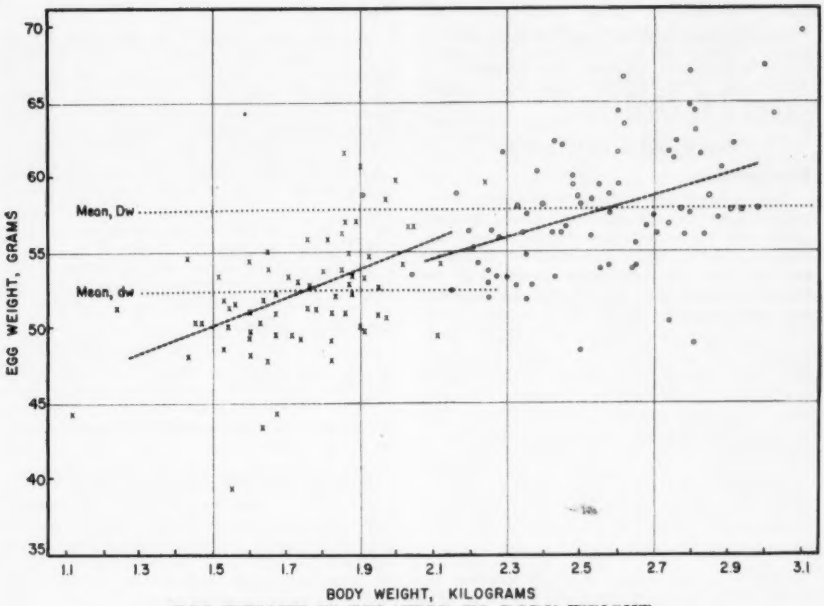
Sire	Coefficient of variability, percent		$\frac{\text{Egg weight}}{\text{Body weight}} \times 100$	
	Dw—	*dw*—	*Dw*—	*dw*—
J 6	7.45	7.52	2.25	2.98
J 7	5.85	7.34	2.28	2.83
J 8	5.94	7.43	2.42	3.13
J 9	5.02	6.02	2.36	2.97

*Numbers of birds as shown in Figure 9.

To study that relationship more precisely, egg weights were plotted against body weights for the 68 dwarfs and their 77 big sisters from ♂ J 6 (Figure 10). Mr. Keith Thompson of the Biometrics Unit of Cornell's Department of Plant Breeding kindly studied the data to determine whether the distributions fitted best to straight lines or to second-degree curves. He found not only that the distributions conform as well to straight lines as to curves, but also that the slopes of these lines are much the same for both *Dw*— and *dw*— females:

$$\text{For } Dw\text{—}: Y = 39.93 + 6.97\,X$$
$$\text{For } dw\text{—}: Y = 35.54 + 9.51\,X$$

In other words, there is nothing unusual about the relatively large eggs laid by the dwarfs. They merely illustrate in a range of smaller birds a relationship between egg size and body size that is equally operative in their larger sisters. This relationship has been reviewed elsewhere[4]. Egg size does increase with



EGG WEIGHT IN RELATION TO BODY WEIGHT

Figure 10

Distributions of egg weight and body weight for dwarfs (x) and their big sisters and half-sisters, all from ♂ J 6. Similar slopes of the lines fitted to these data indicate that the relation of egg size to body size in dwarfs differs little from that in larger birds.

body size, but not directly, and smaller birds tend to lay eggs that are large in relation to body size, whether they carry *dw* or not.

Contrary to expectation, neither of these two distributions shows in itself the curvilinear relationship found by Huxley[6] to apply to Heinroth's data on egg weights in different species within orders of birds. A similar curvilinear correlation whereby relative egg size decreased noticeably in the larger birds was found by Marble[11] in White Leghorns.

Effects on Economic Value

News of dwarf hens reputed to lay large eggs caused some enthusiastic speculation in a farm paper over the possibility that dwarf hens, like dwarf maize, might fill an important niche in an increasingly efficient agriculture. This seems unlikely. As the dwarfs lay not only fewer eggs than their big sisters (Table VI) but also smaller ones, their total output of egg material must be significantly lower. The actual figures, computed from mean production per bird (Table VI) and mean weight of egg per bird (Figure 9) are given in Table VIII. As these comparisons show, the output per dwarf hen during three months of maximum productivity,—March, April, and May—, ranged from 21 to 28 percent below that of her big sisters in four sire families.

Such a reduced output would still have economic value if food consumption of the dwarfs were correspondingly reduced. Precise data on that point are not available, but, as small animals use proportionately more of their feed for maintenance than large ones do, it seems doubtful that the dwarfs could excel their big sisters in the conversion of feed to eggs.

Nine dwarf pullets from different flocks of a strain of White Leghorns known to lay comparatively large eggs were obtained through kindness of Marshall's Hatchery of Ithaca. From preliminary tests and information from other sources, it is believed that these dwarf Leghorns are caused by the sex-linked mutation considered in this paper (Figure 7*B*).

Average definitive egg size for these nine dwarf Leghorns,— 59 grams—, was considerably higher than the mean weights (ranging from 49.2 to 54.7 grams) shown in Figure 5. However, after exclusion of one hen which laid eggs of 66 to 76 grams (but only 40 of them in 110 days), the average egg weight for eight hens dropped to 57.5.

While that figure is remarkable because the average weight of the eight hens was only 1210 grams, these dwarfs laid at a rate of only 39 percent (of the egg-a-day maximum possible),

during 110 days of spring when other birds were shelling out eggs at a 70 percent clip or better. Altogether it would seem that unless better evidence is adduced, the prospect for dwarf hens in the highly competitive poultry industry is not a bright one.

Reproduction and Viability

Reproduction

Because most fowls showing so-called thyrogenous dwarfism (an autosomal recessive character) die early and none attain sexual maturity (Upp[13]), it is desirable to emphasize the fact that with sex-linked dwarfism of the kind herein described reproduction and viability are normal in both sexes.

In two matings of dwarf ♂ × dwarf ♀♀, fertility and hatchability of fertile eggs were 97 and 75 percent, respectively, in one, and 94 and 92 percent for 162 eggs incubated from the other. Fertility and hatchability of eggs laid by dwarfs is evidently normal, as is fertility of dwarf males. However, if rate of reproduction be measured as the number of potential offspring per female (*i. e.* eggs laid), the dwarfs do not quite measure up to their big sisters.

TABLE VIII. Comparisons of dwarfs with their big sisters in average total output of egg material per bird (in grams), during three months

Sire	Daughters: Big sisters	Daughters: Dwarfs	Difference: Grams	Difference: Percent
J 6	3930	3042	— 888	22.6
J 7	3875	3056	— 819	21.1
J 8	3978	2857	—1121	28.2
J 9	3718	2924	— 794	21.4

Viability

Because small animals of any kind are at a disadvantage when required to compete with larger ones for feed and shelter, our dwarfs were usually separated from their big siblings soon after the former could be identified. Under these conditions, viability of dwarfs during the period of growth was no lower than that of their normal-sized sisters. The ratio of dwarfs to big sisters at ages when they could be classified was 1 : 1 (Table I), and in birds weighed at 11 to 12 months of age there was even a slight excess of dwarfs (Table IV).

In the most extensive test of adults of the two types, mortality from the time of housing (when about 150 days old) up to 420 days of age was as follows:

	Died, Percent	*Age at death Mean days*
Among 245 dwarfs	10.6	295.0
Among 222 big sisters	14.9	321.4

Evidently this type of dwarfing has no adverse affect on viability.

Comparisons with Other Dwarfed Fowls

As skeletal dimensions are much less variable than body weights, it is desirable to use the former insofar as possible when attempting to compare the effects of the gene *dw* with those of other genes that reduce size in fowl. Any such reduction is more evident in the length of the leg than elsewhere, and, since other investigators have already provided measurements of the femur, tibio-tarsus and tarso-metatarsus for various kinds of dwarfed fowls, the combined lengths of these three bones provide a useful basis for comparisons (Table IX).

Altogether five different kinds of genetic dwarfing are now known in the domestic fowl.

1. *Normal sex dimorphism* in size usually leaves adult females weighing 19 to 23 percent

TABLE IX. Comparative effects of five different kinds of genetic reduction in size on combined length of the femur, tibio-tarsus and tarso-metatarsus

Kind and source	Combined length of three bones: Larger birds	mm.	Smaller birds	mm.	Reduction: In mm.	Reduction: Percent
1. Normal sex dimorphism						
Maw[12], in Sebright Bantams	6 ♂♂	217.8	16 ♀♀	191.1	26.7	12.3
Hutt[8], in Leghorns	36 ♂♂	317.4	53 ♀♀	277.3	40.1	12.6
Maw[12], in Light Brahmas	9 ♂♂	397.4	18 ♀♀	329.9	67.5	17.0
2. Dominant sex-linked genes						
Maw[12], in F_1 females	4 from Brahma ♂ × Sebright ♀	267.7	7 from Sebright ♂ × Brahma ♀	242.0	25.7	9.6
3. Creeper, *Cp*						
Landauer[10], in brothers	39 *cp cp*	309.2	26 *Cp cp*	226.2	83.0	27.5
Landauer[9a], in females	9 *cp cp*	263.1	26 *Cp cp*	208.5	54.6	20.7
4. Sex-linked dwarfism, *dw*						
In brothers	7 *Dw dw*	392.0	12 *dw dw*	264.1	127.9	32.6
5. Thyrogenous dwarfism, *td*						
Landauer[8], in females	1 *Td Td*	317.9	3 *td td*	193.7	124.2	39.1

*Tables 3, 4 and 28.

less than their full-grown brothers. Data in Table IX show that combined length of the three long leg bones was found to be 12.3 to 17 percent shorter in females than in males, when the comparisons were made in small, medium and large breeds. As poulardes and capons do not differ from normal fowls in size of the skeleton, reduced size of female fowls is not a secondary sex character. In the light of present knowledge it can only be attributed to sex-linked quantitative genes and to the fact that males, with two sex chromosomes, must have twice as many of these as the hemizygous females. As will be shown later, sex dimorphism reduces the lengths of all three long bones more uniformly than any of the three mutants listed in Table IX.

2. *Dominant sex-linked genes* reducing size were proven by Maw[12] to be located in the sex chromosome of Golden Sebright Bantams. In reciprocal crosses between that breed and Light Brahmas, F_1 (gold) females from the Sebright sire were over nine percent shorter in the three long leg bones than the silver females from the reciprocal cross. Other evidence showed that in the F_2 generation gold birds (having the Sebright sex chromosome) were smaller than silvers in both sexes. It is not clear that this dwarfing is caused by any single mutant gene. It is possible that the Sebrights are bantams because of sex-linked quantitative genes *depressing* size and that some of these might even be alleles of the sex-linked genes responsible for normal sex dimorphism in size in larger breeds.

3. *Creeper, Cp.* As Landauer's data[10,9] show, this dominant, lethal gene reduces the three long leg bones of heterozygotes by about 27 percent in males and 21 percent in females.

4. *Sex-linked dwarfism, dw,* reduces these same bones in homozygous males by about 32 percent. Skeletal measurements have not been made for dwarf females, but, since their body weights are reduced by 28 to 32 percent (Table IV) whereas dwarf males weigh 37 to 46 percent less than normal (Table III), reduction of the three long bones in dwarf females is not likely to exceed 24 percent.

5. *Thyrogenous dwarfism, td.* This condition, which was described in detail by Landauer[8] and shown by Upp[13] to be a simple, autosomal recessive mutation, differs from all those described above in being lethal to most of the birds that show it. The few that survive apparently never become sexually mature. The mutation causes brachycephaly, distortion of the sacrum, and reduction of the three long leg bones by about 39 percent in females.

Differing Effects on Individual Bones

As the data in Table IX show, the gene *dw* shortens the leg somewhat more in dwarfed males than the *Cp* gene does in viable creepers, but the effects of these two kinds of dwarfing on individual bones are dissimilar. The reductions of these in normal females, in sex-linked dwarfs and in creepers are given in Table X.

As might be expected from the fact that it is more variable in normal fowls than any other bone in the appendicular skeleton[3], the tarso-metatarsus shows the greatest proportionate reduction in all three kinds of dwarfing. Sex dimorphism shortens the long bones in normal females more uniformly than do the other two types. In creepers the femur is reduced relatively less than the two bones distal to it, and the tibio-tarsus is shortened in almost the same degree as the tarso-metatarsus. In sex-linked dwarfs the femur is also shortened less than the other two bones, but it is reduced more than in creepers, and the tibio-tarsus is less affected than the tarso-metatarsus.

The net effects of these differing genetic influences on the proportions of the three long bones in the limb are shown by the following ratios of the two distal bones to the proximal one when length of the femur was taken as 100.

	$\frac{\text{Tibio-tarsus} \times 100}{\text{Femur}}$	$\frac{\text{Tarso-metatarsus} \times 100}{\text{Femur}}$
In normal Leghorn males[3]	144.3	99.2
In normal males, (*Dw dw*)	148.6	100.6
In normal Leghorn females[3]	141.7	95.5
In dwarf males, *dw dw*	132.1	84.1
In creeper males, *Cp cp*[10]	127.0	92.1

Linkage

From a number of tests for linkage it has been possible to locate the gene *dw* fairly accurately in the sex chromosome. Most of these tests were made with heterozygous males mated to females not dwarfed, and therefore crossing-over could be measured only in the female progeny.

To save space, and because a revision and extension of the map for the fowl's sex chromosome (involving *dw* and other genes) is in preparation, detailed data are omitted here.

Cumulative results of linkage tests over several years were as follows:

	Gametes classified, number	*Crossing-over percent*
dw and *S* (silver)	686	7.0
dw and *K* (slow feathering)	970	6.6
dw and *B* (barring)	359	55.4
K and *S*	977	2.8

These data show that *dw* is remote from *B* and close to *K* and *S*. They put *K* much closer to *S* than earlier tests had indicated[4], but Jaap[7] has recently reported finding only 1.3 percent

crossing-over between these two genes. From all considerations (including some unpublished data) it seems probable that the order of these five genes is *B—S—K—dw*.

Discussion

Any lengthy review of the voluminous literature on dwarfs of divers kinds and species seems unnecessary in this report. There are dwarf races and strains, the results of natural or artificial selection, and representing the cumulative action of many genes affecting size. There is disproportionate dwarfing caused by mutations that induce various kinds and degrees of achondroplasia. Genetic disorders of the endocrine glands cause fowls, mice, rabbits and guinea pigs to be sickly, stunted and sterile. One of them may be responsible for the so-called "proportional" or ateleiotic dwarfs in man, some of whom are apparently sterile.

None of these genetic reductions in size seems comparable to the dwarfism described in this report. It is remarkable not only for the degree of reduction caused by a single mutation in homozygous males and in hemizygous females, but also because there are apparently no effects whatever in heterozygotes. It seems odd that such a powerful mutation should be so completely recessive.

The fact that this sex-linked dwarfing has been found in both New Hampshires and White Leghorns suggests that the mutation may not be an uncommon one. It might be discarded in modern commercial flocks because of the poultryman's general aversion to "runts" of any kind. On the other hand the dwarfs are so obviously vigorous, and in many respects so attractive, that some breeders would preserve and multiply them as curiosities. It would not be surprising if the gene *dw* should eventually be found in some of the many breeds of bantam fowls.

Summary

A sex-linked recessive gene, *dw*, was found to reduce body weights of homozygous males by about 43 percent below normal and those of hemizygous females by 26 to 32 percent.

Chicks that later become dwarfs are normal in size if hatched from eggs of normal size, but by two weeks of age retardation of growth has begun. Dwarfs grow at a slower rate than their big siblings, but without any abrupt cessation of growth up to 26 weeks, at least. Those hatched from small eggs remain smaller for many weeks than those hatched from big eggs.

Viability, fertility and hatchability of eggs are as good in these dwarfs as in larger birds. However, in four sire families, egg production of 249 dwarf females was 7.9 to 13.3 percent below that for their 222 big sisters and half-sisters. Average egg size for the dwarfs was 4.8 to 6.5 grams smaller than those for their big sisters.

Effects of this type of dwarfing on the skeleton, as shown by lengths of the three long bones of the leg, are compared with those of four other kinds of genetic dwarfing in the fowl. All three bones are shortened more, and more uniformly, in dwarfs than in creepers.

The gene *dw* is remote from *B* (barring) and shows crossing-over of 6.6 percent with *K* (slow feathering), and 7.0 percent with *S* (silver). The probable order of these genes in the chromosome is *B—S—K—dw*.

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TABLE X. Reduction of individual leg bones by three different kinds of dwarfing

Item	Femur	Tibio-tarsus	Tarso-metatarsus
In 7 *Dw dw* males, ave. length, mm.	112.4	166.8	112.8
In 12 *dw dw* males, ave. length, mm.	83.7	110.1	70.3
Reduction in dwarfs, mm.	28.7	56.7	42.5
Reduction, percent, in dwarf males	25.5	34.0	37.7
Reduction, percent, in normal Leghorn females*	11.1	12.7	14.2
Reduction, percent, in Creeper males†	19.3	29.3	30.9
Reduction, percent, in Creeper females†	13.4	23.1	24.4

*As compared with Leghorn males; calculated from data of Hutt[3]
†From Landauer[8] (p. 32)

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NO TIME FOR CUNCTATION

NEITHER the slow and placid ox nor the bellowing, easily-frightened steer is any novelty whatever, and the castration of bulls to provide draft animals and milder, tenderer and juicier beef has the sanction of centuries.

But there is an ominous note, if a somewhat devious one, in the British story of the ultimate disposition of cow-faced Brook Mandore, specifically an Ayrshire "breeding bull" whose normal proclivities were banned—whose very life, indeed, was threatened—by the Ministry of Agriculture. Why? Because the sweet-visaged creature was by nature gentle, tractable and, above all, harmless. The Ministry did not wish to perpetuate his genes.

The British have for generations been a dauntless people, and not merely dauntless. Aggressive, even bellicose, no less—symbolized by the lion, the bulldog and, until recently, by pro-Empire colonialism. So belligerant and over-bearing were they that the American colonies, not without courage themselves, decided as long ago as 1776 to part company from them—in the interests of life, liberty and the pursuit of happiness (denied to Brook Mandore), and to preserve internal peace.

Nearly all Americans today agree peace is not only desirable, but essential for the world's security—in fact, for humanity's continued existence. Belligerancy is not, therefore, a quality which should be preserved, in either cattle or men. Most Americans probably would assert that, if bulls can be bred to be gentle and refined, so much the better—even though most Americans are chiefly familiar with the bull found on TV programs, rather than the bucolic type.

British insistence upon the animal's more primitive characteristics is, therefore, to be deplored.

But there is an even more sinister aspect of the Brook Mandore story, if you will permit me to analyze it for you. There is, at the present time—throughout the world—what has been termed a "population explosion," directly affecting not cattle, but men. Various methods of birth control have been suggested to cope with this appalling population increase, which is becoming a menace of the first order as we humans out-produce the earth's readily obtainable food supplies, and even living space. This is directly related to the case of Brook Mandore, as you can see, for he had been destined, before the Ministry of Agriculture interfered, to be a breeding bull.

The method used in the sad case of Brook Mandore has not, overtly, been recommended—not indiscriminately, at any rate—for the human species. But there is no telling when it may be. Especially as Prime Minister Harold Macmillan, whose government was so overwhelmingly re-elected as a Conservative force—God save the mark!—now plans to

(*Continued on page* 247)

CYTOGENETICS OF FOUR SETS OF TWINS IN COTTON

J. E. Endrizzi*

THE occurrence of polyembryonic seed is not uncommon in the New World cottons (genus *Gossypium*). The frequency of twins, however, differs within and between species of the genus.

Haploid/diploid twins have been reported to be by far the most common type in cotton, and essentially it is from this type of twins that haploids have been recovered. Once haploids are recovered, their chromosome number can be doubled with colchicine to produce theoretically homozygous lines that are not only useful in breeding, but also useful in physiological experiments. The importance of doubled haploids in agronomic research has been emphasized in the past by several investigators, and more recently, Harland[5] has discussed in detail the many uses for doubled haploids in the elucidation of genetic problems and in practical plant breeding in cotton. A number of doubled haploids in cotton have already been produced and used extensively in various research programs.

Even though there are several reports in cotton dealing with twin seedlings, very little is known about the origin of the twin embryos. The present report, which is the first of its kind in cotton, covers the cytogenetics of four sets of twins. Unlike the twins previously reported in cotton, three of the four twins were recovered from crosses between parents that were not only different genetically, but were also different cytologically. Therefore, cytogenetic analysis of these twins has provided basic data for a better understanding of the origin of the twin embryos in cotton.

Materials and Methods

Four sets of twins were recovered from 1086 seeds germinated singly in six ounce paper cups. Most of the 1086 germinated seeds were the progeny of hybrids involving the amphidiploid species, *Gossypium hirsutum*, and three diploid species, *G. arboreum*, *G. herbaceum* and *G. thurberi*, which had been backcrossed two or more times to various lines of *hirsutum*. Included in the *hirsutum* lines were genetically and cytologically marked stocks. A minor portion of the seeds consisted of cytogenetic lines of *hirsutum* and a few derivatives from the hybrid *hirsutum* × *barbadense*.

A brief pedigree and the cytological and allelic constitution of the parents for each set of twins are given below. The female parent is listed first in the three cases where twins were recovered from a cross:

Twin Set #1:

Z1951: 24II + chain 3; *arboreum-thurberi-hirsutum* hybrid backcrossed twice to *hirsutum*; *Yy* (heterozygous yellow petal); *pp* (cream pollen); plant hairs absent, but progeny segregated for the character; seed almost naked of fuzz; leaves small, three lobed, due to monosomic condition.

2B-1: 24II + homozygous translocation; Coker 100; *yy* (cream petal); plant hairs absent of the type observed in Z1951 progenies; leaves large, five lobed.

Twin Set #2:

Z1951: As above.

Z1859: 24II + ring of 4; *hirsutum-herbaceum* hexaploid backcrossed twice to *hirsutum* to restore tetraploid *2n* condition; *yy* (cream

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petal); *Pp* (heterozygous yellow pollen); plant hairs absent; heavy coat of seed fuzz; leaves large, five lobed.

Twin Set #3:

Texas 531: 26II; *hirsutum* marker line; *yy* (cream petal); *pp* (cream pollen); h_2h_2 (glabrous stem); *nn* (fuzzy seed); shallow lobed leaves.

Z2261: 24II + ring of 4; *arboreum-thurberi-hirsutum* hybrid backcrossed several times to *hirsutum*; H_2h_2 (heterozygous pilose); *Nn* (heterozygous naked seed); deeply lobed leaves.

Twin Set #4: from selfed seed of Z2320, 26II; *hirsutum*; R_1r_1 (heterozygous red plant body); Lc_1lc_1 (heterozygous brown lint).

Yellow petal, yellow pollen, plant hairs (H_2) and naked seed (*N*) behave as simple independent Mendelian factors. Linkage between any of these factors and the interchanges has not been determined. The plant hairs and seed fuzz characters in Z1951 do not behave in a segregating population as simple Mendelian units similar to the two related factors above. There is a definite association of the three-lobed leaves and the monosomic condition of Z1951. The mode of inheritance of the deeply lobed leaves of Z2261 has not been determined.

Twin seedlings were separated by washing the roots free of soil. This served not only to determine whether the twins were conjoined, but also as an additional check on their origin from a single seed. In each case the twins were found growing side by side, but not physically joined; their secondary roots originated at parallel positions. At this stage the twins were separated and transplanted to the field.

Frequent detailed observations were made on a number of morphological characters in addition to those listed in Table I. Materials for cytological studies were killed, fixed and stained, following the conventional iron-aceto-carmine squash technique. Cytological data were recovered from metaphase I cells of the parents and the twins.

Results

Four sets of unattached twins were recovered from 1086 germinated seeds. Three of the twin pairs were diploid/diploid and one was haploid/diploid. In the young seedling stage two sets had plants which were strikingly unequal in size and it was assumed at the time that they were haploid/diploids. However, the smaller member of one pair later proved to be a diploid and soon grew to be about equal to its partner in size. The three diploid/diploid twins were obtained from a cross of two lines that were different cytologically and genetically (Table I), while the haploid/diploid pair was from self pollinated material (Table II). In two of the three diploid/diploid sets (#2 and #3) the twins were identical, cytologically and genetically, while in the other pair, the two members were different in both their cytological and morphological characteristics.

The cytogenetic aspects of the parents and set of twins are given below.

Set #1: Only two cytological types have been recovered in outcrosses with the chain of three in Z1951. The 2B-1 parent was homozygous for a reciprocal translocation. Therefore, upon crossing the two aberrant chromosome types only two cytological classes are expected. In the analysis of the progeny of this cross the two classes were the only ones recovered, and these are represented in twin plants Z2425 and Z2426 (Table I). Excluding in Z1951 the leaf lobing character, which is highly associated with the monosomic condition, the two parents also differ in three phenotypic characters. Assuming that there is no linkage of the interchanges and the three plant characters, it is therefore theoretically possible to recover 16 cytophenotypes in the progeny of Z1951 × 2B-1. Two of the 16 classes were recovered in the twins (Table I). Since two classes were recovered and both include cytological and genetical characters from the two parents, it follows that the pair was the re-

sult of fertilization of separate cells in the female parent.

Set #2: The male parent (Z1859) of this pair of diploid twins was heterozygous for a reciprocal translocation. Gametes resulting from alternate disjunction of the ring of four are usually the only functional types from the male parent, therefore, this parent produces functional gametes of two cytological types. As was discussed under Set #1, the chain of three in Z1951, which was also the female parent in Set #2, produces cytologically two kinds of gametes. Consequently, it is possible in the progeny of Z1951 × Z1859 to recover four cytological types. Omitting the leaf lobing character for reasons pointed out earlier, the two parents differ also in four phenotypic characters. Therefore, there are potentially 64 cytophenotypes that may be recovered in the offspring of this cross. Only one of the 64 types was recovered in the twins, i.e., both members were identical. In Table I it may appear that the twins were not hybrids, but were wholly of maternal origin. If this had been the case, their origin would require an abnormal cell division that would eliminate the factor for yellow pollen and at the same time retain the chain of three chromosomes, a process which is highly unlikely. Furthermore, the twins had other plant characters similar to their sibs which were unmistakedly hybrids.

Set #3: The female parent (Texas 531) of this set of diploid/diploid twins had the normal chromosome arrangement of *hirsutum*. The male parent (Z2261) carried in addition to the ring of four a pair of chromosomes with the *arboreum* chromosome end arrangement which is different from that in *hirsutum*. Gametes arising from alternate disjunction of the ring of four are generally the only male types which are functional. Consequentally, Z2261 produces functional gametes of two cytological types. Details of the chromosome complex of Z2261 are irrevelent for the present discussion other than to note that when this complex is crossed to Texas 531 two cytological types are recovered in the offspring, one being 24 bivalents and a ring of four and the other being 23 bivalents and a ring of six.

In addition to their cytological dissimilarity, the two parents were also different in five phenotypic characters. Therefore, it was theoretically possible to recover in their progeny 64 cytophenotypes. Both members of this set of twnis were identical in their cytological and morphological characters (Table I). Their cytological composition was of the kind which can be

TABLE I. Cytology and genetics of parents and diploid-diploid twins

		Cytotype	Genotype				
			Petal color	Pollen color	Plant hairs	Seed fuzz	Leaf lobing
Parents	♀♀ Z1951	24II + Chain 3	*Yy*		"+"*	—*	3
	♂♂ 2B-1	24II + TT†	*yy*		—	+	5
Twin set #1	Z2425	23II + I + uneq. 4	*Yy*		—	—	3
	Z2426	24II + uneq. 4	*Yy*		+	—	3
Parents	♀♀ Z1951	24II + Chain 3	*Yy*	*pp*	"+"	—	3
	♂♂ Z1859	24II + Ring 4	*yy*	*Pp*	—	+	5
Twin set #2	Z2427	24II + Chain 3	*yy*	*pp*	—	—	3
	Z2428	24II + Chain 3	*yy*	*pp*	—	—	3
Parents	♀♀ T531	26II	*yy*	*pp*	h_2h_2	*nn*	Shallow
	♂♂ Z2261	24II + Ring 4	*Yy*	*Pp*	H_2h_2	*Nn*	Deep
Twin set #3	Z2380	23II + Ring 6	*yy*	*pp*	h_2h_2	*Nn*	Deep
	Z2400	23II + Ring 6	*yy*	*pp*	h_2h_2	*Nn*	Deep

*"+" = plant hairs not conspicuous in parent but factors present for the character; + = present, — = absent; signs used for those characters whose segregation do not fit a simple Mendelian ratio.

†TT = homozygous translocation.

obtained only from a combination of the two parents, thus proving that the twins were hybrids.

Set #4: This set of twins was haploid/diploid and they were recovered from selfing Z2320. The parent was heterozygous at two loci (Table II) which determine the plant characters red plant body (R_1) and brown lint (Lc_1). Both of these factors are incomplete dominants; therefore, nine different phenotypic or genotypic classes may be distinguished in the progeny of Z2320.

The diploid member was phenotypically homozygous for both dominant genes, while the haploid was phenotypically hemizygous for the R_1 allele (Table II). The lint color of the haploid could not be determined since haploids of *hirsutum* are completely male and female sterile. It was pointed out above that this pair of twins was recovered from selfing a plant which was heterozygous at two loci. Since the diploid member was homozygous at the two loci and the other member was a haploid, then it is evident that both members were sexual in origin.

Discussion

The cytogenetic study of four sets of twins in cotton showed that three sets were diploid/diploid and one was haploid/diploid. The data further revealed that all members of the diploid/diploid sets of twins had both maternal and paternal characters, but that members of one of the diploid/diploid set of twins had different cytophenotypes, while members of each set of the other two pairs of diploid/diploid twins had identical cytophenotypes. In the haploid/diploid set the data on the diploid member showed that the diploid, like the haploid, was sexual in origin.

A detailed review of polyembryony in plants has been given by Webber[7]. In general he has classified them as follows: (a) sporophytic budding, (b) cleavage, i.e., the separation of the zygote or young embryo into two or more units, (c) simple polyembryony, or the result of the development of more than one egg in a single embryo sac; this also includes the formation of egg-like synergids or antipodals, and (d) the development of more than one embryo sac in an ovule. There are variations within the above types, but they need not be considered in this discussion.

Development of the embryo sac in cotton follows what is known as the normal type, i.e., following two successive divisions of the megaspore mother cell, one of the four megaspores develops into an eight-nucleate embryo sac.

The presence of more than one embryo sac in an ovule has been favored as an explanation of the origin of twins in cotton[4,6]. However, except for the single case (Set #1) reported here, there is no example in the literature of twin seedlings in cotton whose analysis clearly points to their origin from two embryo sacs within a single ovule.

Only two studies of sectioned ovules in cotton have been reported in which deviations from the normal type were found. In the earliest of the two studies, Baranov[1] reported finding an occasional production of two embryo sacs within a single cotton ovule. Apparently, the assumption that twins arose from separate embryo sacs had its stimulus from Baranov's[1] findings. In the second report, Quintanilha, Cabral and Quintanilha[8] found that among a few hundred ovules that were examined, there were two with embryo sacs containing 16 nuclei instead of the usual eight. They suggested that such types of embryo sacs could give rise to the kinds of twin seedlings reported in cotton.

Twin plants from Set #1 in the present report were different cytologically and genetically, and their cytological difference was due entirely to the female parent since the male parent was homozygous for an interchange. Therefore it is most likely that each member developed from separate embryo sacs. An additional possibility is that the plural seedlings were derived from fused ovules. This type of twinning has been reported by Weber[7] to be common in cotton.

In the case of twin sets #2 and #3, where the pairs of each set were identical and both sets arose from hybridization of the two parents, it seems most logical to assume that they were the results of cleavage of a zygote. On the other hand, it is conceivable that they could have arisen from a 16-nucleate embryo sac or from egg-like synergids. However, this would require that the two eggs in a 16-nucleate embryo sac or the egg and the egg-like synergid in a single embryo sac be fertilized by the two sperm from a single pollen tube. In this connection, the observations of Quintanilha *et al.*[8] in one of their abnormal embryo sacs is of interest in that when the two male nuclei entered the sac, one fused with one egg-cell and the other fused with one pair of polar nuclei, leaving one egg unfertilized. This observation has some bearing on the origin of the haploid/diploid twins.

TABLE II. Cytology and genetics of parent and haploid-diploid twin pair

			Genotype	
		Cytology	Plant color	Lint color
Parent	Z2320	26II	R_1r_1	Lc_1lc_1
Twin set #4	Z2444	Haploid	R_1	
	Z2445	26II	R_1R_1	Lc_1Lc_1

The data on the haploid/diploid set studied in this experiment were somewhat limited, therefore, the origin of this pair of twins is less conclusive than in the previous cases. This pair of twins was recovered from selfing a plant that was heterozygous at two loci. Since the diploid member was homozygous at the two loci, this then rules out sporophytic budding as being involved in the origin of the diploid member. Therefore it seems conclusive that this set of twins arose from one or two embryo sacs. Since both members of the haploid/diploid pair carried the R_1 allele it seems most probable that only one embryo sac was involved and that perhaps it was equivalent to the 16-nucleate type of embryo sac described by Quintanilha *et al.*[8]. In this case one of the eggs was fertilized and developed into the diploid twin, while the other egg was not fertilized, but was stimulated to develop into the haploid member.

Summarizing, it was found that only one of the four sets of twins involved dual embryo sacs, while two pairs of twins were undoubtedly from a single embryo sac. It is most probable that the remaining set of twins was also from a single embryo sac. In all other reported cases of twins in cotton the data were insufficient to arrive at any definite conclusion whether one or more embryo sacs were involved. However, there is a single example[6] in the Asiatic cottons of twins which could be ascribed to dual embryo sacs.

Even though the number of twins is small in the present study, it does, however, indicate that twins in cotton may arise more frequently from a single embryo sac than from two as previously assumed.

Cytology of the haploid

In the analysis of the pollen mother cells of the haploid, 116 metaphase I cells were scored for the frequency of paired chromosomes. In nineteen of the 116 cells, paired chromosomes were observed in addition to univalents. Eighteen of these cells had a single one chiasma bivalent, while one had a trivalent configuration. The average association per cell was found to be 25.66 I + 0.16 II + 0.008 III. The average of 0.16 II per cell is much lower than the 1.7 and 1.5 observed in *hirsutum* haploids by Beasley[2] and Brown and Menzel[3] respectively.

Since the chromosome complement of the amphidiploid *hirsutum* is composed of 13 pairs of large A chromosomes and 13 pairs of small D chromosomes, a haploid would contain 13 large A and 13 small D chromosomes. The size difference is evident in metaphase I cells of the haploid, thus making it possible to score the bivalents as to the size of the chromosomes making up the pair. The 18 bivalents were scored according to the size of the chromosomes in each pair, and the results were as follows: 2 = two large chromosome, 3 = large and medium, 11 = large + small, 1 = small + medium, and 1 = small + small or medium. Assuming that most medium size chromosomes are A chromosomes, which is likely, it is evident that most bivalents consisted of pairing between A and D chromosomes.

Metaphase I cells in the present haploid were typical of those reported in other haploids where the univalent chromosomes were observed to be scattered throughout the spindle. One cell, however, was found with all 26 univalents oriented on the equatorial plate.

Summary

A cytogenetic study was made on four sets of twins in cotton. Three pairs were diploid/diploid, while the other pair was haploid/diploid. Only one set of twins arose from dual embryo sacs, and two were undoubtedly from a single embryo sac. It is most probable that the remaining set of twins, the haploid/diploid pair, was also from a single embryo sac. An average of 0.16 bivalents per cell was found in the haploid of *Gossypium hirsutum*.

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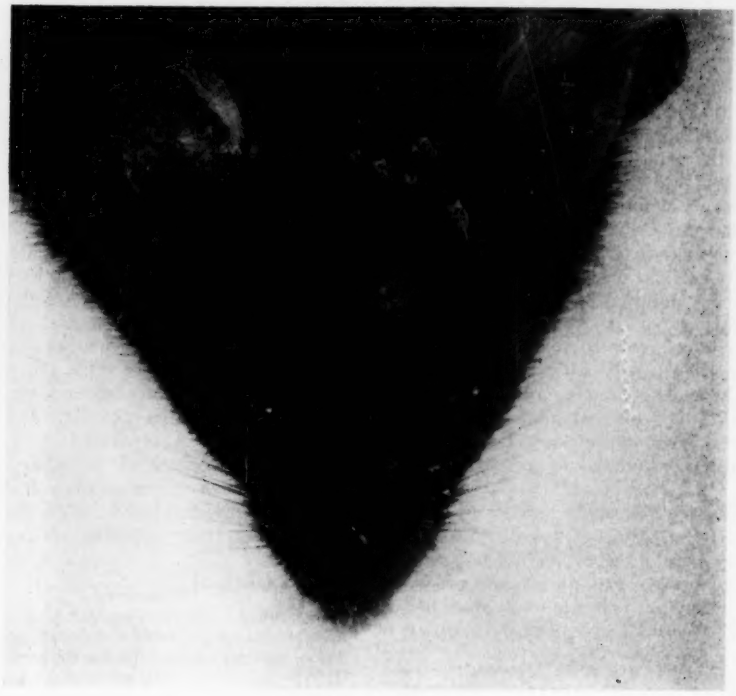
TUFTED — A NON-GENETIC MORPHOLOGICAL VARIANT IN MICE

of a Polydactylous Descent

LEONELL C. STRONG AND LEONARD ROMAN*



A TUFTED VARIANT

Figure 11

A polydactylous female mouse showing the light tan tuft of hair on the head. The hair surrounding the tuft as well as that of the rest of the body is of medium brown.

TUFTED, the rare morphological variant reported here (four cases), is characterized by a light tan tufted growth of hair on the forehead of an otherwise brown non-agouti mouse of a polydactylous descent when the hair has completed its first growth cycle. Although there is no previous report in the literature of an identical condition in the house mouse, Searle[1] reported a similar variant in the C57 Bl/Gr mice which has interesting similarities and differences to the new "tufted" condition presently reported.

The purpose of this note is to describe the gross aspects, present the genetic find-

* Biological Station of Roswell Park Memorial Institute, Springville, New York. This experiment has been made possible, in part, by a grant (H2666 C) from the National Heart Institute of the U. S. Department of Health, Education and Welfare.

ings, and give a brief description of the histology of the guard-hairs of the "tufted" region.

Methods and Results

This aberrant condition which was observed in a mouse of the polydactylous descent is distinguishable from normal offspring at the time of birth. Newborn which develop this tufted condition have a papilloid growth measuring 2 mm. long by 1 mm. in diameter located at the junction of the parietal and interparietal bones of the skull. As these young mice age, the papilloid growth is either transformed into a small flap of skin or remains as a papilloid growth. It is later obscured by the growth of hair which forms the tuft, (see Figure 11). The hair of the tuft has a light tan color while the hair surrounding the tuft as well as the hair covering the rest of the body is of a medium brown color (genetic constitution *aabbCC*). Examination of the skull of the mouse showing the tufted condition to determine whether there was any other abnormalities associated with it, revealed no cranial or vascular anomalies.

The polydactylous descent of mice in which "tufted" arose, has ancestors which were treated with methylcholanthrene for many generations. Polydactylia, one of the mutant sublines, was continued as brother-sister matings while selection of these mice was, and is presently, based on parental age[2].

Although both black and brown mice segregate in the polydactylous strain of mice, all tufted mice (three females : one male) were born to four brown females × brown males in a colony of about 1,200 breeding females. To determine whether this variant was a mutant or not, two of the mice with the tufted condition were bred. In one case, the tufted male was mated to his normal sister and the female progeny of this mating were again backcrossed to the tufted male (single backcross), and the females born of this mating were again backcrossed to the tufted male (double backcross). Of these matings in which the male was tufted a total of 86 offspring were born. All were normal. In the second case, the tufted female was mated to her normal brother and a male offspring was backcrossed to the variant parent. Of these backcross matings, 72 normal offspring were born. The results of both matings are summarized in Tables I and II.

Individual hairs from the tuft as well as the surrounding brown hair were examined by the method of Russell[3]. The guard hairs of the tuft as compared to the adjacent brown hair were found to be composed of a long apical region in which the distal end contained neither pigment nor medullary air-cells while the proximal end contained only traces of pigment located mainly in the medulla. The subapical region, not separated by a subapical band, contained medullary air-cells in which there was a reduced amount of pigment; and the cortex contained only trace amounts of pigment granules.

Discussion

The tufted condition observed in the polydactylous descent of mice and the similar condition, crested, reported by Searle in the C57 Bl/Gr mice are of interest from genetic and anatomic aspects. Since the variant condition was seen only four times in litters born to 1200 breeding females in the period of one year, and was not transmitted from parent to offspring, it is apparent that this variant is not a mutant. However, since the polydactylous strain of mice had C57B1 ancestry, the mechanism which is involved in the expression of both variant conditions may possibly be the same. The general appearance and location of the

TABLE I. Results of matings of tufted female mouse and normal male mice

Matings	Normal	Poly	Tufted	Totals
Brother-Sister (normal)(tufted)	3	30	0	33
1st Backcross	5	16	0	21
2nd Backcross	5	13	0	18

TABLE II. Results of matings of tufted male mouse and normal female mice

Matings	Normal	Poly	Tufted	Totals
Brother-Sister (tufted)(normal)	9	24	0	33
1st Backcross	14	5	0	19
2nd Backcross	22	12	0	34

cutaneous proturberance which apparently has the ability to reduce pigmentation in the hair of the tuft appear alike; whether finer differences do exist which will indicate similarity or dissimilarity will remain a matter of speculation until a larger number of mice with this abnormal condition can be obtained.

The four mice with this tufted condition were three females and one male which is indicative that this condition is not sex-linked, while the condition as it occurs in the C57 Bl/Gr appears to be a sex-limited character, since the four mice which were crested were all males.

These data strongly indicate that this variant is neither a mutant, nor associated with sex differences, and is not inherited by known genetic principles. This variant as well as similar variants demonstrated the spontaneous origin as well as the sporadic appearance of phenotypes which may involve extra-nuclear inheritance.

Summary

The rare variant designated as tufted was observed in a colony consisting of black and brown polydactylous mice; the four tufted mice (3♀ : 1♂) as well as the normal parents were all brown.

Tufted is distinguishable from normal newborns by a characteristic papilloid growth located on the forehead which later may develop into a flap of skin. The cutaneous growth is obscured by the light tan tuft of hair which grows from it. There are no skeletal anomalies nor sterility associated with this condition.

A tufted female was mated to her brother, their progeny backcrossed and then double backcrossed, which was the same procedure of mating employed for the tufted male. Of these matings a total of 158 offspring were born, none expressing this variant condition.

These data strongly indicate that this variant condition is not a mutant, is not associated with sex, and is a non-genetic manifestation but is apparently dependent in some subtle manner upon the genetic background of the C57 strain.

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WE REGRET THE DELAYS

Every effort is being made on put the *Journal* back on a current publication basis. However it will be several months before this can be fully achieved.

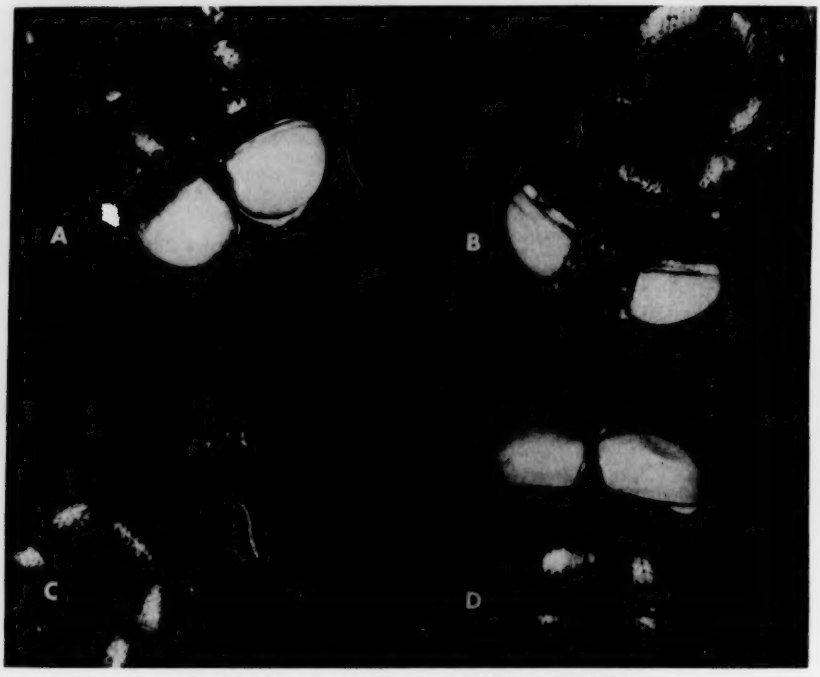
During the past year we have been beset with difficulties of many kinds, which are being slowly but surely rectified.

To members, subscribers, and authors who have submitted manuscripts for publication we ask your patience for a little while longer. A great deal of progress has been made in the business of catching-up and there is no reason at present to suppose that it will not continue. But we still have quite a long way to go.—B.C.L.

A NEW MUTANT OF PHORMIA REGINA

And its Allelic Relations

CARL S. HAMMEN*



EYE-COLOR MUTANTS OF *PHORMIA REGINA*
Figure 12

A and *B*—White mutants; *C*—wild type (dark red-brown); *D*—yellow. (*Photograph by John Cuculis*).

THE blow fly *Phormia regina* has been and continues to be the subject of numerous physiological investigations on chemoreception, respiration, and nutrition. Dichler[1] described a "white-eyed" mutation which was white on emergence but which assumed a yellow or pink tinge with age. This mutant was probably identical with the yellow-eyed strain which is maintained by the entomology laboratory at the Army Chemical Center and which was investigated biochemically by Ward and Hammen[2]. From the yellow strain there occasionally have appeared flies with permanently snow-white eyes, differing from the pale yellow of the majority at emergence and even more from the darker yellow of aged individuals (Figure 12). Some results of attempts to determine the genetical and biochemical character of this new white mutant are reported here.

Attempts to Establish a Breeding Stock

As white-eyed flies emerged from culture jars of yellow pupae they were iso-

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lated and an attempt was made to start a colony with two to six adults of each sex. These produced numerous batches of eggs which failed to hatch. Also seven single pairs were mated, and these had no offspring. Finally, when whites were obtained in the F_2 of other crosses as many as 35 of each sex were held together and a total of 70 pupae were obtained. From these puparia there emerged 11 adults and 15 near-adults which died with only the heads protruding, all of which had white eyes, suggesting that the strain is true-breeding.

Hybridization Experiments

Crosses of white females with wild-type and yellow males failed because of early death of the females or non-hatching eggs. White males, however, were capable of fertilizing wild-type and yellow females. In the cross of white × yellow 21 pupae yielded 13 adults, all yellow. The F_2 from these F_1 yellows amounted to 155 pupae from which were obtained only 16 yellow and eight white. These results are consistent with the hypothesis of yellow dominance over white.

Wild is dominant over yellow, giving good 3:1 F_2 ratios, as was shown by Dichler[1]. Therefore it was not surprising to find that the cross of white male × wild-type female (eight pairs mass-mated) gave all wild-type offspring (97 flies from 107 pupae). In the F_2 of this cross, however, there appeared not only both parental types but yellows as well. The numbers of each type and sex are presented in Table I.

The females show fair agreement with the hypothesis of a 12:3:1 dihybrid ratio. On the basis of a 1:1 expected sex ratio, which is actually shown by the yellows, there is a serious deficiency of wild and white males. Since yellow ordinarily breeds true, it could not be the heterozygote of wild and white, nor can its presence be explained by the existence of three alleles. Most probably there are two pairs of alleles, and the genotypes represented in this F_2 are: *yyww* is white, a double recessive; *yy W-* is yellow, a single recessive; *Y---* is wild-type, an epistatic dominant. The semantic evolution of the term "epistasis" was traced by Hollander[2], and this case seems to fall within the definition he suggested, "the masking of the phenotype of a mutant or non-standard gene by that of another, not an allele."

Body size of the strains

When the counting of offspring of experimental crosses was nearly complete, 30 to 50 newly emerged flies of each sex and each phenotype were weighed in order to check the impression of reduced body size of the mutants. The results (Table II) suggest that the yellows are 15 percent smaller than the wild-type, and the whites about 30 percent. Unfortunately, the variation was such

TABLE I. F_2 of white male × wild-type female

	wild	yellow	white	total
A. Females				
actual	2,358	638	188	3,184
expected*	2,388	597	199	
deviation	−30	+41	−11	$\chi^2 = 3.80$
d^2/e	0.38	2.81	0.61	$0.20>P>0.10$
B. Males				
actual	2,188	635	164	
expected	2,240	560	187	2,987
deviation	−52	+75	−23	$\chi^2 = 14.09$
d^2/e	1.21	10.05	2.83	$P < 0.01$
C. Total				
actual	4,546	1,273	352	6,171
expected	4,628	1,157	386	
deviation	−82	+116	−34	$\chi^2 = 16.06$
d^2/e	1.45	11.61	3.00	$P < 0.01$

*Expected numbers calculated on the hypothesis of a 12:3:1 dihydrid ratio.

that the size of the sample proved to be too small to achieve statistical significance, and there was no opportunity to repeat the measurement on additional flies.

Tryptophan analyses

Ward and Hammen[3] showed that the yellow-eyed mutant strain has the same defect in tryptophan metabolism as that of the mutant cinnabar of *Drosophila melanogaster*, resulting in an accumulation of kynurenine in the body fluids. Table III gives the results of free tryptophan analyses made on extracts of the lyophilized whole bodies of newly emerged flies. Two batches of each strain were analyzed. The Horn and Jones test shows little variation among them, while the Eckert test, less specific for tryptophan, indicates that a substance related to tryptophan, probably kynurenine, accumulates in both yellow and white to a level about four times that of the wild-type.

Thus the white mutant has the same metabolic defect as the yellow, and an additional defect preventing synthesis of the yellow pigment. Since the nature of this yellow pigment and its precursors are still unknown, it is not possible now to characterize the presumed additional defect any further. Its pleiotropic effects on fertility and viability are nevertheless clearly deleterious.

TABLE II. Weight of newly emerged adults

Strain	Number of each sex	Mean weight (mg) ± S. E. Females	Males
wild	30	38.5 ± 4.0	35.7 ± 4.4
yellow	50	32.6 ± 2.0	30.5 ± 1.8
white	33	26.5 ± 2.8	25.9 ± 2.4

ADDENDUM:

The yellow-eyed mutant has been investigated further by Bodenstein, whose larval transplantation experiments and injection of pure pigment-precursor metabolites brought him to the same conclusions regarding the metabolic defect. (BODENSTEIN, D. Contributions to the problem of eye pigmentation in insects: Studied by means of intergeneric organ transplantations in Diptera. *Smithsonian Misc. Coll.* 137: 23-41. 1959.)

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TABLE III. Free tryptophan analyses; (mg/g dry weight)

Strain	Horn & Jones test	Eckert test
wild	0.304	0.348
	0.268	0.338
yellow	0.222	1.218
	0.372	1.100
white	0.386	1.464
	0.230	1.224

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CORRIGENDUM

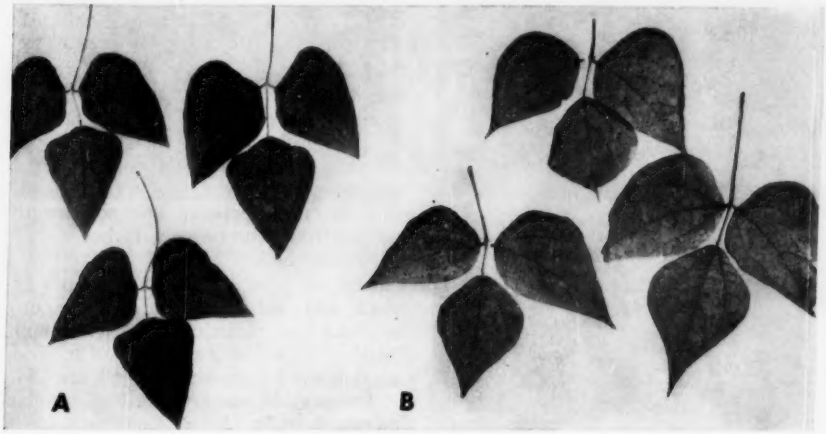
An error appeared in the legend for Figure 17, Page 147 of the article by Blackwell *et al.*, "A Hydrocephalic Lethal in Hereford Cattle," which appeared in the May-June 1959 issue of the *Journal*. The legend as it should read is as follows:

Pedigree showing introduction of lethal gene through sire A and typical matings that produced hydrocephalic calves. Old line mates omitted for simplicity. Squares represent males; circles, females; diamond, sex unknown. Solid shaded symbols represent hydrocephalic calves; solid half-shaded symbols represent parents of hydrocephalic calves; cross-hatched half-shaded symbols represent animals incriminated as carriers through their descendants. The coefficients of relationship between 19 lethals and bulls A and D averaged 21 percent and 38 percent, respectively. The coefficients of relationship among the 19 lethals averaged 26 percent and their inbreeding coefficients averaged 11.7 percent.

The editor regrets the error.

INTERSPECIFIC HYBRID BETWEEN PHASEOLUS VULGARIS AND P. LUNATUS

SHIGEMI HONMA AND OTTO HEECKT*



TYPICAL *PHASEOLUS* LEAVES

Figure 13

A—Shows mature leaves of *Phaseolus lunatus; B*—those of *P. vulgaris.*

WITHIN *Phaseolus vulgaris* there is need for a snap bean combining green cotyledons with a green seed coat color. Various intravarietal crosses have yielded light green seed coat progenies, but none with green cotyledons. The cross between *P. vulgaris* and *P. lunatus,* a lima bean, was attempted to combine a darker green seed color with green cotyledon in the snap bean.

Interspecific crosses in the genus *Phaseolus* have been reviewed elsewhere[3]. Since then, however, Rudorf[6] reported a successful cross using *P. coccineus* as maternal parent with *P. vulgaris.* Numerous investigators have previously reported the reciprocal cross. Wall[7] reported the use of intervarietal F_1 hybrids of one species crossed with a "pure" variety of another was slightly superior to the best variety × variety in obtaining interspecific crosses of *P. vulgaris* × *P. coccineus.* Successful interspecific crosses involving the species *P. vulgaris* × *P. lunatus* to the writer's knowledge have not been reported, although Kooiman[4] reported attempts of crosses were made without success.

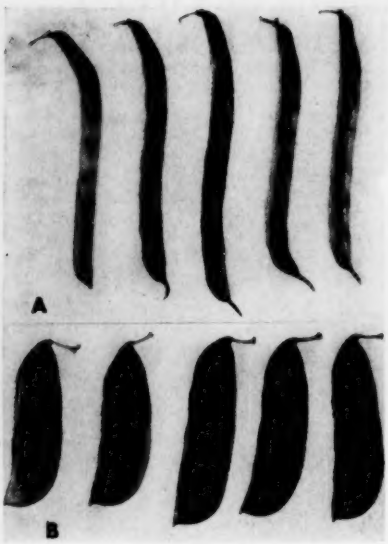
The findings reported here are, of necessity, limited since large populations are difficult to obtain from a small number of interspecific hybrids.

Parental Description

Numerous varieties of *P. vulgaris* and *P. lunatus* were used in the investigation without success. Since no crosses resulted from using pure strains, intraspecific F_1 plants of *P. vulgaris,* (Contender × Blue Lake) F_2 × (Kentucky Wonder × Contender) F_2 and the intraspecific F_2 plants of *P. lunatus* Fordhook × Clark's Bush selection were used.

Morphological differences in characters of these two species are illustrated in Figures 13-15 and Table I. The measurements of leaf shapes were taken

* Michigan Agricultural Experiment Station, Journal Article Number 2371.



POD TYPES
Figure 14
A—Phaseolus vulgaris with round to oval and slender pods with long spurs; *B—P. lunatus* with short flat pods and short spurs.

from field-grown plants. The primary leaves of the lima are darker green in color with distinct veining as compared to indistinct veining and lighter green color of the snap bean. The length of the petioles of the primary leaves are longer in the lima bean than in the snap bean.

The flowers of *P. lunatus* differ from the snap bean in shape as well as color of the floral part. In the snap bean the banner is either white or colored as the rest of the floral parts while in the lima the banner is of a different color from the wings, usually greenish-yellow with a purple tinge. Flowers in the common bean are borne on ends of short peduncles in a close aggregate of clusters, while flowers in the lima bean are borne in racemes at the ends of long peduncles.

The pod of the lima bean is large, thick, broad, and heavy about three to five inches long. Pod margins are thick and have a short, stout, blunt beak, and a spur located to the side of the apex. (Figure 14). Pods of the common bean are slender, straight, or curved, flat or oval in cross section. The pod length ranges from four to eight inches with a prominent beak.

The seeds of *P. lunatus* are flat or thick, and oval with a more or less conspicious line radiating from the hilum. Seeds of *P. vulgaris* are elliptical in cross section and kidney shaped. (Figure 15).

Parental differences noted are similar to that described by Freeman[1] and Hedrick[2] for these two species.

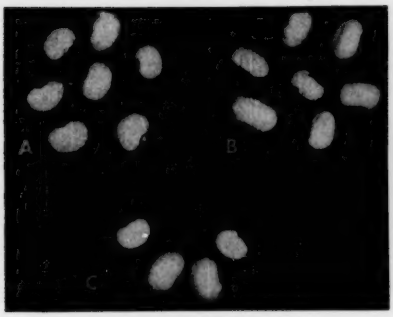
Hybridization

Methods used in pollination were similar to those described by the authors in a previous paper[3]. An unrecorded number of pollinations using several varieties of the two species set pods but generally aborted within seven days. A few remained longer, but no seed developed in the pods. Species hybridization was attempted using heterozygous parental plants. Numerous pollinations using *P. vulgaris* as the female parent yielded two pods with mature seeds. Ten seeds resulting from the cross were grown in the greenhouse. Numerous reciprocal crosses were attempted using pure and

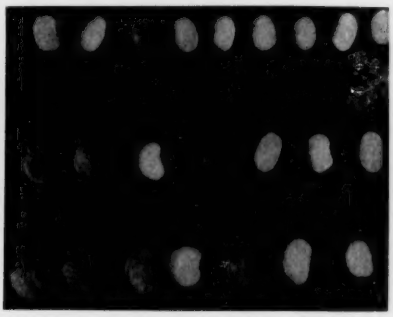
TABLE I. Comparison of true differences between segregates and parents for mature terminal and lateral leaves

Leaflet		*Phaseolus vulgaris*	*Phaseolus lunatus*	Segregate	Range	P*
		Average	Average	Average		
Terminal:	length	8.26 ± 1.22 mm	12.86 ± 1.73 mm	12.87 ± 2.29 mm	6.25 - 19.00 mm	.01
	width	6.42 ± 1.38 mm	7.28 ± 1.02 mm	8.20 ± 2.11 mm	2.45 - 12.57 mm	.34
Ratio:	length/width	1.31 ± .17	1.77 ± .12	1.66 ± .52	1.30 - 4.53	.68
Lateral:	length	7.60 ± 1.57 mm	11.54 ± 1.88 mm	10.73 ± 1.94 mm	4.55 - 15.60 mm	.09
	width	5.75 ± 1.72 mm	6.63 ± 0.96 mm	7.20 ± 1.97 mm	1.90 - 10.77 mm	.45
Ratio:	length/width	1.33 ± .09	1.74 ± .14	1.59 ± .56	1.80 - 4.87	.75

*Possibility of true difference between segregate and *P. vulgaris*.



SEED TYPES
Figure 15
Seeds of *Phaseolus lunatus* (*A*) and *P. vulgaris* (*B*) are shown above. Seed shape of the F_1 (*C*) are intermediate between the two parents.



SEED SHAPES AND SIZES
Figure 16
Seed shapes and sizes in the segregating generation are shown above.

heterozygous plants but no pod development occurred.

F_1 Hybrid Characteristics

The F_1 seedlings together with the parental plants were grown in pots in the greenhouse. The seeds were germinated in a quartz sand medium. As soon as the radicle was approximately one inch in length the seedlings were transplanted to pots of soil. All seedlings reached maturity. Eight of the 10 F_1 hybrids were morphologically similar to the *P. vulgaris* parent with varying degrees of self-fertility. Pod and seed shape of the F_1 plants were intermediate between the two parents. (Figure 15). Two of the progenies were of willow leaf types and were sterile.

Characteristics of the F_2 Generation

Since only a limited number of seeds were obtained from the hybrid plants, notes and illustrations were made from F_2 and F_3 plants. The 112 progenies from the hybrids with the parents were grown in pots in the greenhouse.

Measurements of mature leaf parts were made from 200 F_3 hybrids and the probability of true difference between the hybrid and *P. vulgaris* are shown in Table I. A difference in length of terminal leaves between hybrids and *P. vulgaris* parent is suggested. The P value of .09 for the lateral leaves is suggestive of a difference between the *P. vulgaris* parent and the segregated material. It is of interest to note from the table, the range in leaf sizes obtained from the segregating population. Observations on leaf shape of the F_2 population were similar to that noted in the F_3 population and a representative sample is illustrated in Figure 17.

Variations in mature pod shapes are obvious in Figure 18. Recombination of spur types and pod shapes can be noted. Variations in spur lengths and pod shapes suggest polygenic inheritance for these characters. Seed shapes and sizes are shown in Figure 4. A recombination of colors was noted in the population. The appearance of these colors was expected since heterozygous plants were used to obtain the cross. No attempt was made to factorily analyze the color, shape, or size inheritance. Only one plant yielded a light green seed coat color.

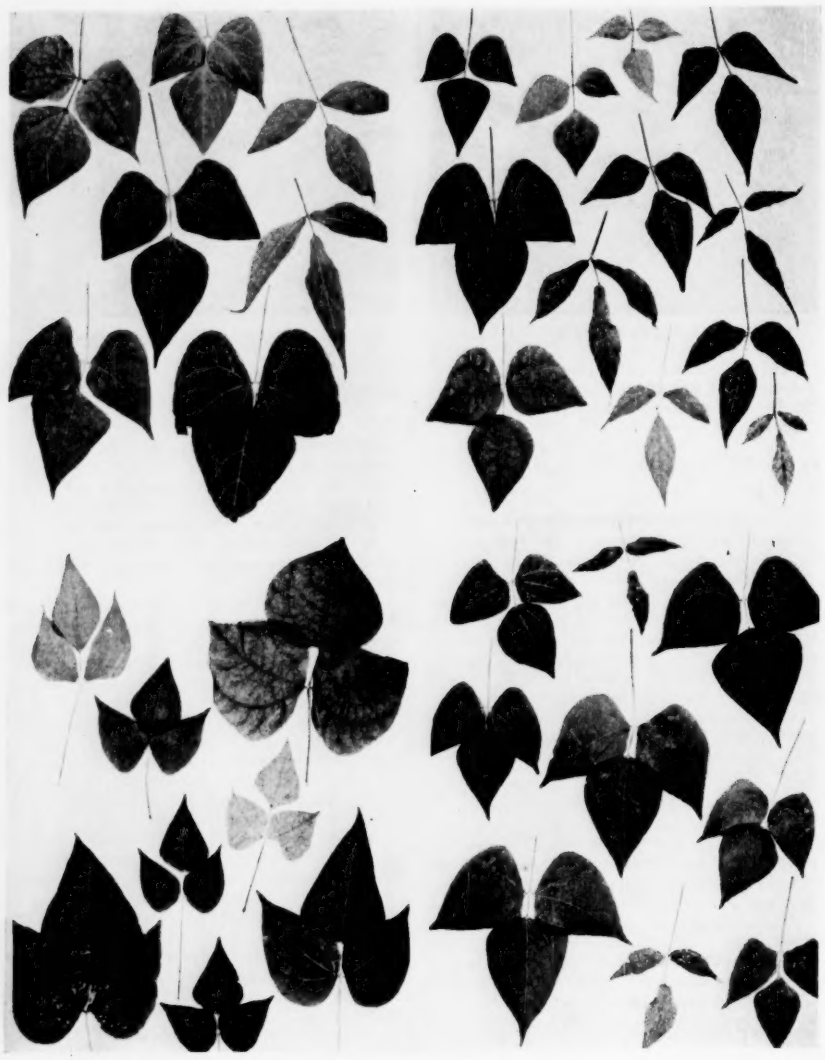
Fertility of the progenies varied as indicated from normal set to no set. Sterile types were generally associated with the crinkle, narrow or willow leaf, virescent plants. Plants with lanceolate leaves, in most cases, failed to survive. These plants died at various stages up to the time of flowering. Few obvate-lanceolate leaf plants dropped their leaves after pod set and failed to produce viable seeds. The amount and degree of floral development of the progenies appeared to be associated with leaf type, i.e., a larger number of normal flowers were produced as the leaf shape approached ovate or normal types. Similar observations were made by Lamprecht[5]. No cytological studies were made to relate the anomalies observed to chromosome deletions as suggested by Lamprecht[5].

Plant types observed ranged from the lima bean type to common bean types. No parental types were recovered from the F_2 or F_3 populations.

Backcross

Success in crossing the parents to the interspecific hybrids varied. Some of the hybrid plants crossed readily to both parents while others failed to set pods. Backcrosses to *P. lunatus* resulted in viable seeds, however, and both normal and abnormal plants also resulted. Crosses with *P. vulgaris* parents resulted in viable seed with fewer abnormal plants than backcrosses with *P. lunatus*. Anomalies observed were similar to those found in F_2 and F_3 populations.

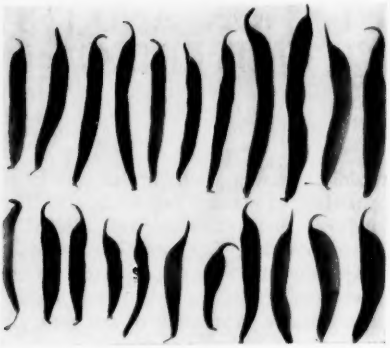
Backcrosses of the F_2 plants, to the *P.*



HYBRID LEAVES

Figure 17

The various leaf types in the segregating generation are shown above.



MATURE HYBRID PODS
Figure 18

Hybrid pod shapes are shown above. Note recombination of size and shape of both parents in the segregating generation.

lunatus parents were not as successful as when *P. vulgaris* plants were used. Only a small number of backcrosses were obtained with *P. lunatus*.

Discussion

The data and figures demonstrate that a hybrid was secured between *P. vulgaris* and *P. lunatus*. It is apparent that the use of gametic diversity aided in making the interspecific cross. Although the lima bean parent failed to carry the desired characteristic green seed coat and green cotyledon in the initial cross, a segregate was found with light green seed coat color. The desired characteristics can be transferred readily by crossing the segregant with a lima bean possessing green seed coat and cotyledons as demonstrated by the backcross to the hybrid.

In the F_2 as well as in the F_3 no parental types were recovered although a few approached the *P. vulgaris* type. It is of interest to note the association of the lethal character with willow leaf type plants and the age of the plant. This observation was also noted in the backcross F_2 and F_3 populations.

Summary

Numerous crosses using heterozygous parents of *P. vulgaris* and *P. lunatus* made it possible to obtain 10 hybrid plants. Eight hybrid plants were similar to the *P. vulgaris* plants while two others failed to resemble any of the parents and appeared as willow-leaf sterile plants. The fertility of the eight plants ranged from low to normal. Backcrosses of both parents to the hybrid were made with little difficulty. Segregation for leaf type, seed and pod shape were noted in the F_2 and F_3 populations. Variations in the characters suggest a quantitative type of inheritance.

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WADDLER, A NEW MUTATION, AND ITS INTERACTION WITH QUIVERING

C. H. Yoon*

A mouse having the condition named "waddler" appeared in the furless stock maintained in the Department of Biology at Lycoming College in 1957. This is the same furless stock which produced a mutation called "quivering" in 1953[1]. During 15 generations of close inbreeding no mice of waddler type had previously been observed. It was, therefore, assumed that this condition was the result of a mutation. Genetic tests showed that the waddler condition depends upon an autosomal recessive gene (gene symbol, *wd*) and that this gene is not allelic with the gene quivering (gene symbol, *qv*). In order to study the interaction between the waddler and quivering genes, mice affected by both of these genes were produced by appropriate matings. These doubly affected mice showed not only symptoms of both waddler and quivering but showed these symptoms in an exaggerated form.

Description

Homozygous waddler mice can be detected as soon as they begin to walk. Definite, unmistakable classification can be made around 14 days of age. As they walk the hindquarters sway from side to side, forming a rather smooth arc and they often fall on their hips when the effect is severe. No trembling motion or paralysis is observable. The locking of hind legs, which is so common among neuromuscular mutants, was also observed in many cases but there was only one case of priapism among a total of 180 waddlers examined. Both males and females are fertile in contrast to the sterility of many similar mutations. Two matings between waddler males and waddler females each produced a litter consisting of nothing but waddlers. The longevity of waddler mice is affected to various degrees. The condition is neither progressive nor does it improve. Consideration of the reproductive performance and the expression of the gene excludes the possibility of repetition of any neuromuscular mutations already reported.

Bodyweight

Both normal and waddler mice from litters of seven were weighed at 21 days, 28 days and 42 days of age. The average bodyweights of these animals are plotted against their age in Figure 19. Both waddler males and females are lighter than their normal sibs (Figure 19*A* and *B*). These differences in body weight between waddler and normal mice are statistically significant, and they increase as the animals grow older. This is shown by the steady upward rise of the curves (Figure 19*C*). The difference in body weights between waddler and normal males becomes abruptly sharper at 28 days. This is due to the fact that many waddler males failed to show any growth during the natural weaning period which is between the third and the fourth week after birth. Evidently normal mice take weaning much more easily than waddler mice. This same tendency was also frequently observed in the case of females when the growth curves of individual waddler females were examined.

Genetics

Data from the breeding experiment are shown in Table I. Eight waddler

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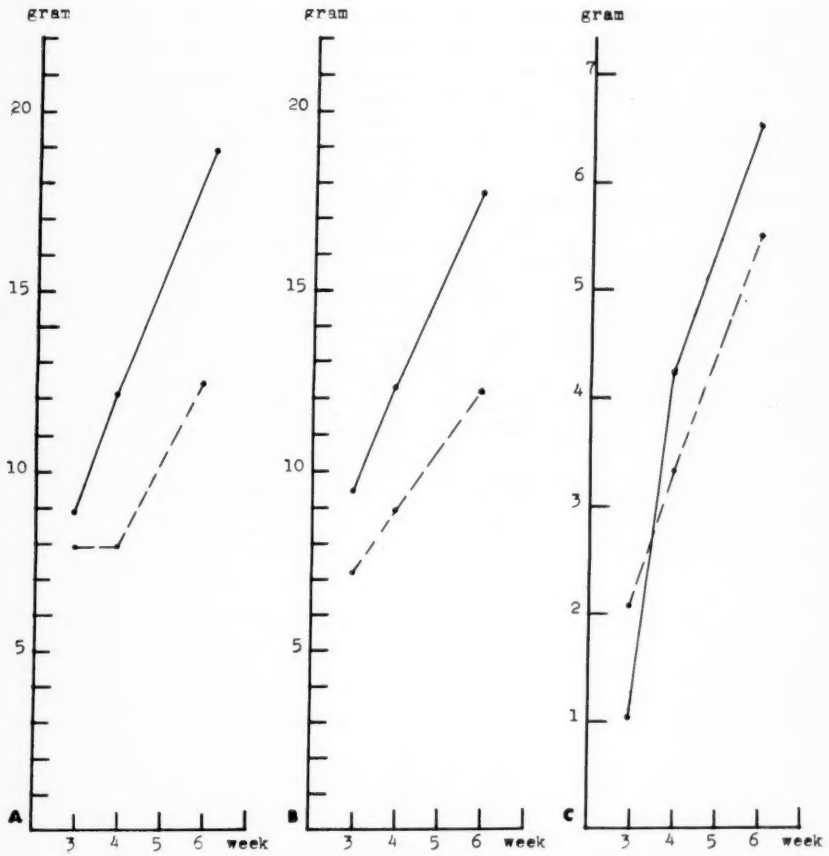


COMPARISON OF GROWTH CURVES OR NORMAL AND WADDLER MICE

Figure 19

A—Bodyweight of normal and waddler males, *B*—of normal and waddler females; C—difference in bodyweight between normal and waddler mice. Solid line = normal mice and broken line = waddler mice in *A* and *B*. Solid line = males and broken line = females in *C*.

males and females were mated to normal males and females from BALB/c, SEC, FS and QV strains of mice. These matings produced 11 litters, consisting of 30 F_1 males and 40 F_1 females. All of these 70 mice were normal. From these F_1 mice 24 matings were made. These matings produced 81 litters, consisting of 373 normal and 108 waddler mice. These numbers do not differ significantly from the expected three to one ratio of normal to waddler, if the waddler condition is due to autosomal recessive genes. As a further test eight waddler males were mated with eight F_1 normal females. These matings produced 14 litters, consisting of 33 normal and 24 waddler mice. These numbers again do not differ significantly from the expected ratio of one normal to one waddler if the same

assumption is true. Finally several matings between waddler males and waddler females were made. Two of these matings produced, one litter each, consisting of a total of 12 mice. All of these mice were waddlers, confirming the simple recessiveness of the condition.

Allelic Test with Quivering Gene

Allelic tests with the quivering gene were made by crossing quivering females with waddler males. Two such matings produced three F_1 males and five F_1 females, all normal. From these mice four matings were made to produce F_2 animals. A total of 25 litters were obtained. The distribution of these four types of mice are shown in Table II. There were 110 normal, 36 waddler, 37 quivering and 13 waddler-quivering (mice affected by both waddler and quivering conditions) mice. These numbers do not differ significantly from the expected ratio of 9:3:3:1, if the waddler and quivering genes assort independently.

Interaction Between Waddler and Quivering

Abnormal motion and bodyweight

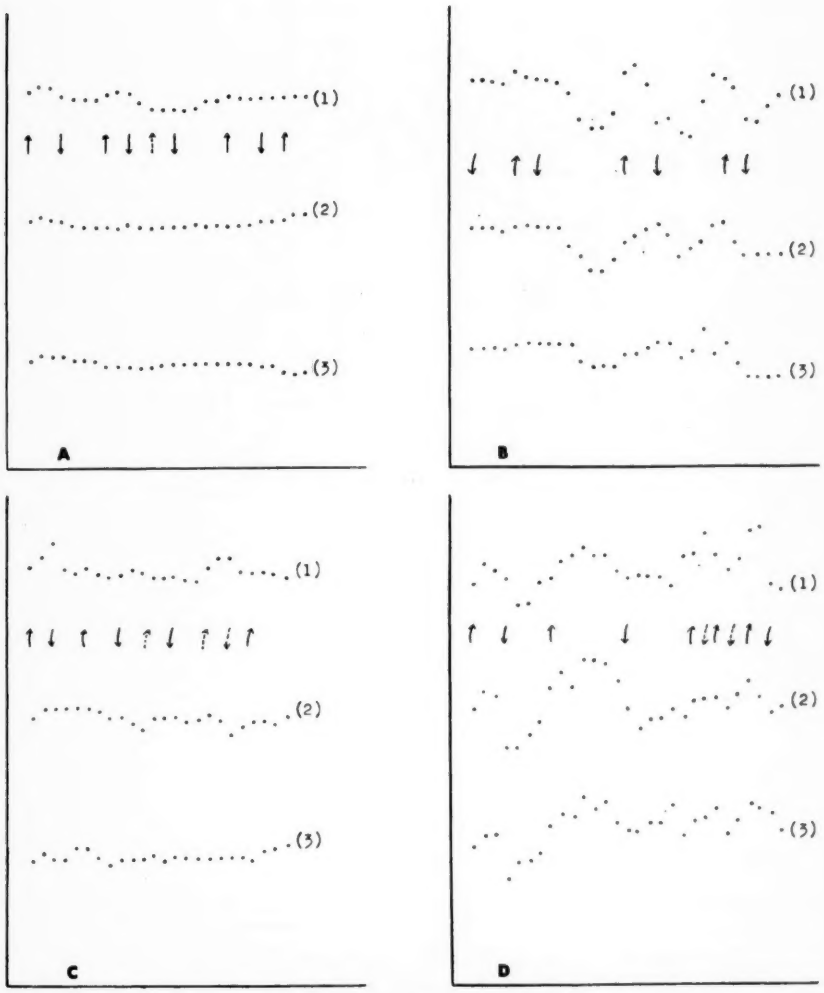
The characteristics of quivering mice were described previously[1]. As is clear from the descriptions of quivering and waddler, these two types of mutations are phenotypically rather different; usually it is not difficult to distinguish one from the other. First of all the degree of swaying in the hindquarters in waddler is much more pronounced than that of quivering. Quivering mice rarely fall on their sides while they walk before weaning, but it is rather common for waddlers, especially when they walk on an uneven surface. While quivering shows a marked trembling during motion or right after the cessation of motion, waddlers do not show this symptom beyond a normal degree. While the quivering condition is invariably progressive, the waddler condition is not progressive. While no quivering male has ever fathered any offspring, the fertility of waddler males is only slightly lower than that of normal brothers when mated with normal mice. The waddler-quivering mice, affected by both waddler and quivering conditions, are

TABLE I. Results from mating waddler with normal mice from BALB/c, SEC, FS and QV strains

Mating Sire	Dam	Normal ♂	Normal ♀	Waddler ♂	Waddler ♀	Total
Waddler	× Normal	21	30	0	0	51
Normal	× Waddler	9	10	0	0	19
		30	40			70
F_1	× F_1	193	180	64	44	
Observed F_2		373		108		481
Expected 3:1		360.75		120.25		
		$\chi^2 = 1.66$, df. $= 1$, $P > 5\%$				
Waddler	× F_1 normal	13	20	17	7	
Observed B_1		33		24		57
Expected 1:1		28.5		28.5		
		$\chi^2 = 1.42$, df. $= 1$, $P > 5\%$				
Waddler	× Waddler	0	0	4	8	
		0		12		12

TABLE II. Results of allelic test with quivering

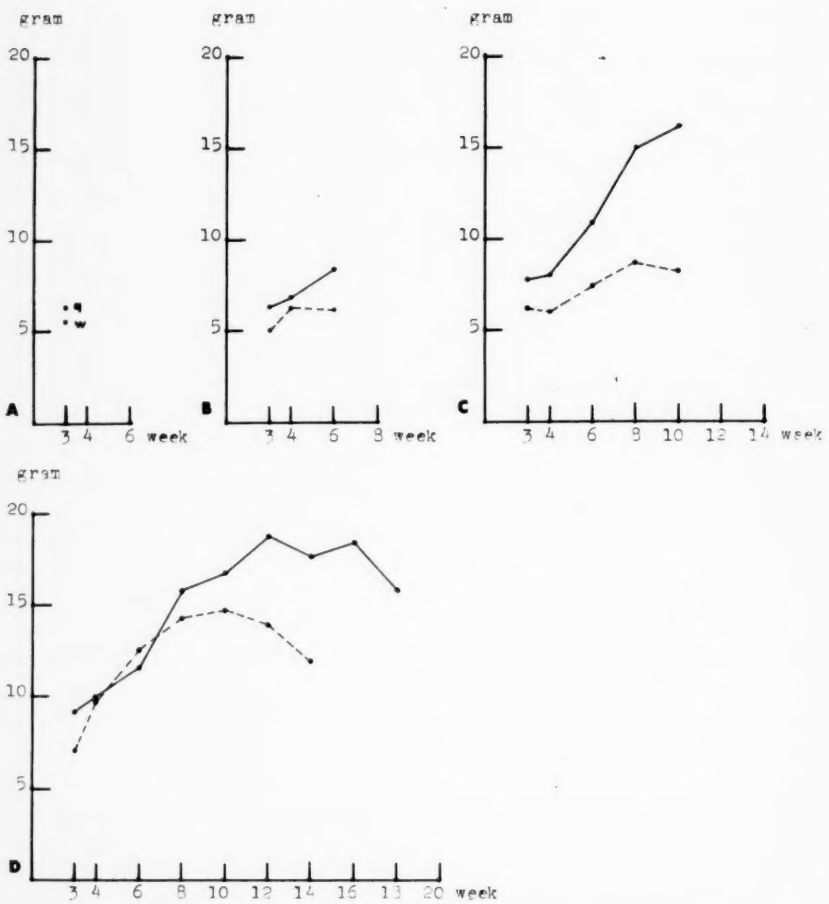
Mating type	F_2 Segregation *QvWd* ♂	*QvWd* ♀	*Qvwd* ♂	*Qvwd* ♀	*qvWd* ♂	*qvWd* ♀	*qvwd* ♂	*qvwd* ♀	Total
QvqvWdwd × *QvqvWdwd*	51	59	19	17	18	19	3	10	91 105
Observed F_2	110		36		37		13		196
Expected 9:3:3:1	110.25		36.75		36.75		12.25		
	$\chi^2 = 0.05$, df. $= 3$, $P > 5\%$								



HORIZONTAL DISPLACEMENT OF THREE REGIONS OF THE BODY

Figure 20

Displacement of a point on the vertebral column at pelvic region, (1); of a point between ears, (2); and of a point on nose, (3). Pictures were taken directly from above the bodies of mice. The magnitude of displacement corresponds to the actual distance. An interval between dots corresponds to 1/32 second. Arrows indicate the direction of the slant of hindquarters at the corresponding moments. Broken arrows indicate the absence of such slanting where it is expected. Arched arrows indicate the presence of twisting posture in addition to slanting. *A*—normal; *B*—waddler; *C*—quivering; *D*—waddler-quivering.



COMPARISON OF GROWTH CURVES OF QUIVERING AND WADDLER-QUIVERING

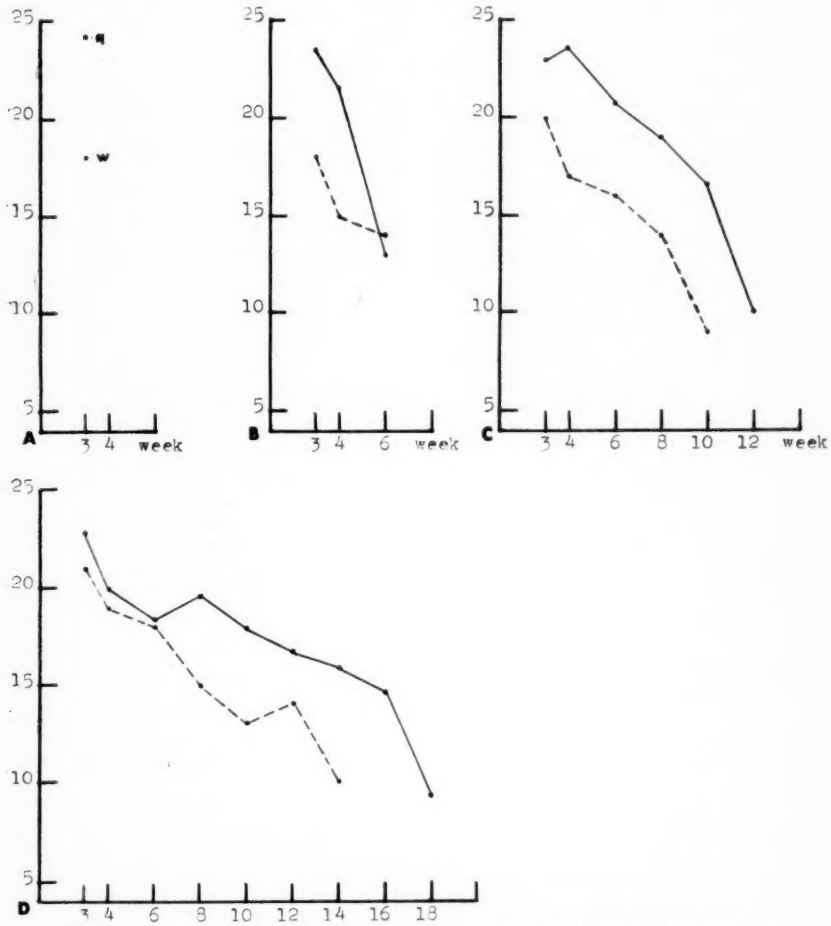
Figure 21

A—Growth curves of mice which died before the fourth week; *B*—mice which died after the fourth but before the eighth week; *C*—mice which died after the eighth but before the 14th week; *D*—and of mice which died after the 14th but before the 20th week. Solid line = quivering; broken line = waddler-quivering.

phenotypically different from those mice having only the waddler or the quivering condition.

They show symptoms of both mutations in an exaggerated form. Their hindquarters sway from side to side like waddlers but with a wider horizontal displacement and their bodies tremble as quivering mice during walking as well as immediately after the cessation of walking. As they grow older the paralysis in the hind legs becomes progressively severe as in quivering mice.

Motion pictures of normal, waddler, quivering and waddler-quivering mice (all about two months old) were taken. Then the horizontal displacement of the three points of the body, a point right above the nose, a point between the ears and a point on the vertebral column at the pelvic region, were traced in each case to



DEGREE OF EXPRESSION OF QUIVERING AND WADDLER-QUIVERING

Figure 22

A—Degree of expression of mice which died before the fourth week; *B*—of mice which died after the fourth but before the eighth week; *C*—of mice which died after the eighth but before the 14th week; *D*—and of mice which died after the 14th but before the 20th week. The maximum score is 29 indicating freedom from effect with a theoretical minimum of zero. The lower scores indicate a greater severity of effect. Solid line = quivering; broken line = waddler-quivering.

show the degree and types of displacement of these points in four types of mice while in motion on a straight line. These tracings are shown in Figure 20. In normal mice there is very little displacement of hindquarters. In quivering mice this hindquarter displacement is still slight. In both waddler and waddler-quivering the hindquarter displacement often reaches such an extent that mice cannot bring the point back to the normal position, falling instead on their sides.

In normal mice the displacement of nose and between ears points is so slight that it is scarcely noticeable. In quivering mice two independent motions are observed at these points; motion accompanying the general movement of the body as a whole which is really a swaying motion and trembling motion in-

dependent of this swaying motion. As shown in Figure 20C the displacement due to the swaying motion of quivering mice at these points is not significantly greater than that of normal mice. Although the trembling motion at these points is clear in direct observation, it is not detectable in the illustrations. This is interpreted as being due to the fact that in trembling motion the nose and neck (between ears) move more vertically than horizontally and accordingly photographs taken directly from above the body of the animal do not reveal the displacement accompanying the trembling. In waddler mice the trembling motion is absent but the swaying motion at these points is indicated by a wide displacement. In waddler-quivering mice the swaying motion at these points is exaggerated. In addition in these mice there are some indications of displacement due to trembling, especially in nose point, as may be seen in the fact that there are extraneous displacement cycles in this point which are not found in the displacement cycles of hindquarters.

Figure 21 shows the comparison of bodyweights between quivering and waddler-quivering mice. It is evident that the forms of growth curves of these two types of mice are very similar, but the growth curves of waddler-quivering mice consistently fall below those of quivering. It was not possible, however, to test the significance of the difference in bodyweight between quivering and waddler-quivering due to the small sample size of the latter.

Effects of waddler gene upon the degree of expression in quivering

It is possible to grade the severity of the degree of expression in quivering mice quantitatively by grading their various symptoms[2]. The degree of quivering, ability to use hind legs, flexion of toes, degree of straightening ability when placed on the back, mobility when freed, ability to walk on narrow bars specially designed for the test, and absence or presence of locking-hind legs when picked up by tails, were graded depending upon the degree of expression, giving lower grades to cases where the degree of expression is greater. According to this system a normal mouse is given a score of 29 which is the maximum score to be attained.

Mice were examined at 21 days and 28 days of age and once every two weeks thereafter until death. Then, mice which died at a similar age were grouped together and the averages of their degree of expression at various stages were computed. Figure 22 shows curves constructed from such averages for several groups of mice. The severity of expression of both quivering and waddler-quivering increases as they grow older as is evidenced by the rather steep decline of the curves. The curves for waddler-quivering are always slightly lower than those for quivering in all the groups, indicating that waddler genes, when combined with quivering mice, accentuate the symptoms of quivering mice. However, again no reliable statistical test can be made because of the small size of waddler-quivering data.

Summary

A new mutation named waddler (gene symbol, *wd*) was found to depend upon an autosomal recessive gene. The condition of waddler mice is described. Consideration of reproductive performance and observable expression of gene effects excludes the possibility of repetition of any neuro-muscular mutations already reported. It is shown that the gene, *wd* is not allelic with the gene, *qv*. The neuromuscular symptoms of waddler, quivering, and waddler-quivering are compared. It has been found that all three types of mice are phenotypically different, showing different types of abnormal motions. The growth curves of normal, waddler, quivering and waddler-quivering mice are compared. It seems that the waddler genes, when present in a homozygous condition, accentuate the degree of expression in quivering.

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CHROMOSOME NUMBER IN THE MINK

DONALD G. HUMPHREY AND NEIL SPENCER*

THE chromosomes of the mink (*Mustela vision,* Peale and Beauvois) have been recently investigated by Shackelford and Wipf[4] who report a diploid number of 28 for the species, and by Lande[2] who has found the diploid number to be 30 for the same species. The paper of Shackelford and Wipf has had a wider circulation and is referred to by Makino[3] in listing the characteristic chromosome complement for this species and also by King[1] in regard to the genetics of mink. This paper was also reprinted in the American Fur Breeder[5]. Shackelford and Wipf used standard dark color ranch-bred mink while Lande used both ranch-bred wild type and mutant Royal pastel. In order to determine which of these reports is correct and in order to investigate the possibility of geographical or other variation in the chromosome number of this species the authors undertook a new investigation of the chromosomes of mink.

Materials and Methods

The mink used in this study came from a population at the Jefferson Fur Farm located in Jefferson, Oregon. Mr. T. W. Riggle kindly donated 10 prime animals including wild mink from central Canada, ranch-bred wild type, mutant Royal Sapphire, Blue Sapphire and Aleutian. The animals were killed by quick suffocation and the testes removed immediately. Parts of testicular material were minced and pretreated in distilled water for intervals of 10 to 30 minutes. Cell suspensions were made and squashed in aceto-orcein. The preparations were made permanent and then studied for suitable mitotic and meiotic figures. Since the animals were taken during the breeding season in March both spermatogonial mitoses and meiotic figures were abundant. Chromosome counts were made on both mitotic metaphases and prophase and metaphase figures of the first meiotic division. Only well-spread figures were recorded, at least 20 counts were made for each animal, and both haploid and diploid numbers were determined. Photographs were taken on 4×5 Ortho Contrast film with an AO Spencer 97X oil immersion objective and a Leitz 12X periplan ocular. A Leitz Zeemf drawing device was used to make drawings of each chromosome set recorded.

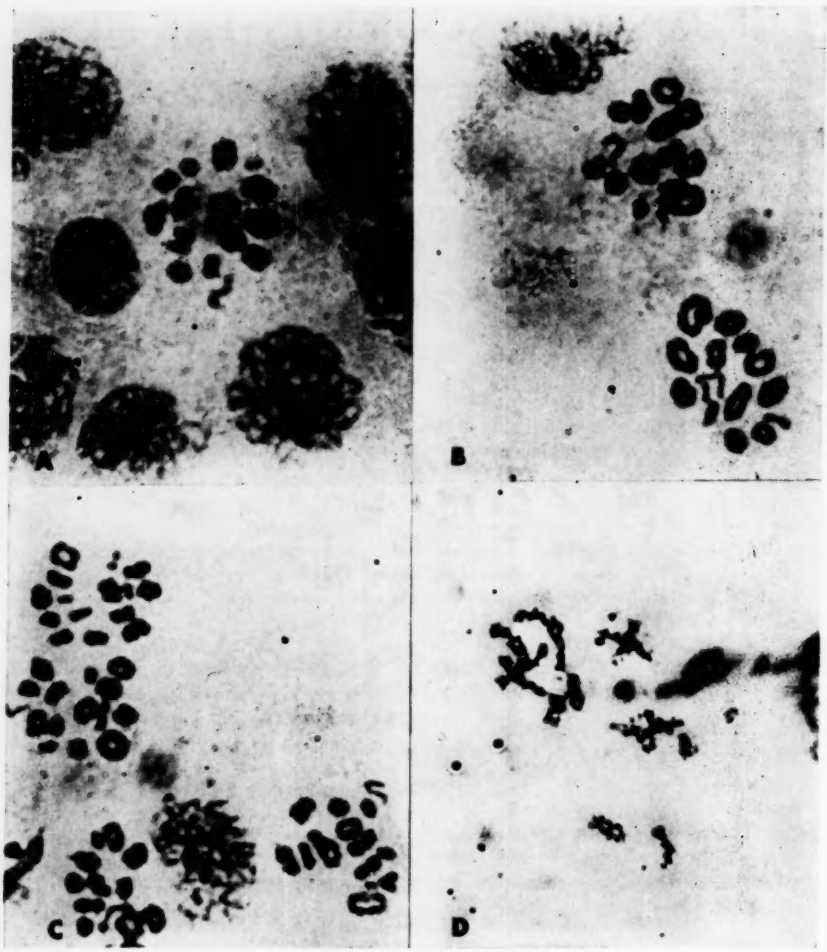
Results

From more than 200 chromosome counts a haploid number of 15 and a diploid number of 30 was determined for the mink. No variation from this number was found in any of the 10 animals studied. In every way the results obtained here agree with those of Lande. Fourteen ordinary bivalents and the probable XY complex described by Lande appear in Figure 23. The mitotic set can be formalized as 6M, 2m, 3S, 3s, and a heteromorphic XY complex; where M = metacentric pairs, S = submetacentric pairs, and capital letters denote the larger chromosomes in the set. Two of the submetacentric pairs might be considered acrocentric. The X chromosome appears acrocentric also. A definite location of the centromere of the Y chromosome is not apparent.

Discussion

This study indicates that the correct number of chromosomes for the mink is $n = 15$; $2n = 30$. This supports the work of Lande and not the work of Shackelford and Wipf. It is doubtful that the latters' results were due to actual variation of chromosome numbers within the species. Lande reports no variation in the five animals studied by him, and in the 10 investigated here no variation was observed. Since the animals included in this work and that of Lande include specimens from Scandinavia, Canada, and the United States as well as

* Oregon State College, Corvallis. This research was supported in part by a grant from the Graduate Council of Oregon State College.



THE CHROMOSOMES OF MINK

Figure 23

A-C—Meiotic metaphase I revealing 15 bivalents, including the heteromorphic XY complex. *D*—Diplotene of meiotic prophase I also showing 15 bivalents.

wild, Ranch and mutant types it would seem that any possibility of geographic or mutational variation is excluded. It is quite possible that the results of Shackelford and Wipf were due to the difficulty of obtaining good material from this species. The pretreatment squash technique employed here overcomes most of the difficulties and insures more definite results. It is hoped that this contribution will clear up the discrepancy in the literature over the chromosome number in mink.

Summary

The chromosomes of mink have been investigated in order to resolve differences reported by earlier workers. The results of this study confirm the work of Lande. The chromosome number for mink is $n = 15$, $2n = 30$, with no

significant variation normally appearing. The earlier report by Shackelford and Wipf of $n = 14$; $2n = 28$ is in error, probably due to the difficulty of obtaining good material from this species by the techniques employed. The male is the heterogametic sex and an evident XY complex is present.

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◆

No Time for Cunctation

(*Continued from page 221*)

legalize gambling and do all manner of radical things in a country already more than half socialistic.

And even this does not convey the enormity we may all face. The castration of Brook Mandore, poor once-male creature, was ordered by the publisher of a British newspaper, The London Daily Mirror. Cynically, The Mirror, said a dispatch from London, "broke the news gently to its readers"—after the deed was done. The emasculated bull, the paper declared, was given "a tranquilizer drug beforehand," and "a local anaesthetic for the operation."

Now, perhaps, the world will realize what newspaper publishers (and not only the British) are capable of doing to humanity, as well as bulls. This is no joking matter—no time for cunctation, procrastination, or any other form of delay—it is time for men everywhere, if they are men, to act. We should have a rallying cry, perhaps even paraphrasing Karl Marx, and saying, "Males of the world unite, you have nothing to lose but your—" But this is too grim, too grim. I simply cannot go on.

Joseph Cloud

Washington, D. C.

A REVERSE MUTATION IN THE HOUSE MOUSE

JOHN DAHLBERG*

ABOUT two years ago, a white-bellied agouti mutation occurred in the strong colony of Brpb (genetic constitution *aabbCCss*) inbred mice. The ancestors of this mutant had received a powerful carcinogen, methylcholanthrene, for successive generations. The origin of the Brpb stock is described by Strong[6] and by Strong and Hardy[7].

The mutant has a "diluted" brown agouti dorsum and a light cream colored ventrum which varies considerably with the individual. Since the mutant was agouti, and since the stock from which it arose was not, it was immediately suspected that the mutant was A^w, since our white-bellied agouti and A^w (in other laboratories) are practically identical.

Little and Hummel[3], in 1947, reported an almost identical situation, but from DBA stock. Evidence was given in this paper to support the truly allelomorphic series of A^y, A^w, etc., instead of loose linkage of the agouti gene with the white-belly gene. In addition, several other instances of A^w mutations have been reported; among them the papers of Little[4] in 1916, in which he first reported an orange-bellied mouse (mentioned later), Engels[1] in 1948 in which a wild A^w mutation was obtained, and those mentioned by Grüneberg[2].

Since the non-agouti gene (*a*) has mutated several times to A^w, it is more than possible that this new white-bellied mutation is A^w. The Brpb is a brown non-agouti piebald animal. The mutant is an agouti light-bellied animal with the dorsum lightened presumably by a modifying gene. Since the dorsum of the Brpb stock is not light colored, it is thought that the modifying gene causing the lightening may be an allele of the gene for color (*C*). It has been established[5] that the gene for chinchilla has very little effect upon non-agouti but lightens agouti to a considerable extent.

It is the purpose of this experiment (1) to show that the new white-bellied mutation is a reverse mutation from *a* to A^w, and (2) to show that the modifying gene acting upon the dorsum of the new mutation in chinchilla, which had been carried along in the Brpb stock before the occurrence of the mutation.

Materials and Methods

All the mice used in this experiment were raised in this laboratory and are of known pedigreed stocks. The laboratory temperature is maintained within a range of 68-73° Fahrenheit with a humidity of about 50 percent. The mice are housed in wooden boxes with metal hoppers and are provided with cotton and kilndried sawdust for nesting materials. A diet of mouse pellets (Mouse Blox) and water is provided *ad libitum* with weekly supplements of mixed grains with cod-liver oil and milk-soaked bread.

The mutant, in addition to its use in the establishment of an inbred strain, has been outcrossed to mice of six other stocks: AKH, Strong A, BrS, C, C57, and C3H. In addition to these basic outcrosses, several supplementary crosses have been made to test the ideas and theories brought up by the data obtained from the various outcrosses. In all of these outcrosses, closeness of fit tests have been performed to demonstrate significance.

*Biological Station of Roswell Park Memorial Institute, Springville, New York. Submitted as part of the trainee program of the Biological Station of Roswell Park Memorial Institute in cooperation with Griffith Institute and Central School, Springville, N. Y. Financed in part by a grant from the National Science Foundation. The author wishes to acknowledge the assistance and advice of Dr. Leonell C. Strong and Mr. L. B. Hardy and extends to them his great appreciation.

Results

From all of the outcrosses 5,772 F_2 animals have been raised and classified. In the outcross to Strong A mice a total of 1,292 animals have been raised, (see Table I). This experiment was conducted to establish a pattern of behavior for the genes of the mutant. The F_1 was a white-bellied animal with much the same dorsum as the mutant parent, i.e., light brown agouti. The F_2 generation segregated into two classes, "light and dark" agouti, (in an approximate ratio of 2:1), dark-bellied brown non-agouti non-mutant, and albinos, in the classic 9:3:4 two gene ratio with an excellent probability of 0.92 (above .05 being significant). To determine the genetic make-up of the two white-bellied classes, two of each class were outcrossed to albino mice, (see Table II).

In the next outcross (Table III) the mutant was mated to mice of the C stock (genetic constitution *AAbCC*). In the F_2 generation brown agouti white belly, brown agouti normal-belly, light brown agouti white-belly, and light brown agouti normal-belly segregated in a typical 9:3:3:1 (two gene) ratio. (The modifying gene acting upon the dorsum shall provisionally be called chinchilla for convenience, as it has not yet been convincingly proven.)

In the tables, the words mutant and

TABLE I. Results of the F_2 of the outcross of the new mutant to the Strong A strain

Mutant			
Light	458		
Dark	261	719	
Brown non-agouti		251	
Albino		322	
Total number		1292	
Observed ratio:		719 : 251 : 322	
Expected ratio: (9:3:4)		727 : 242 : 323	
Chi square: .40177	Probability: 0.92		

Genetic constitution of parents and F_1 generation*
Mutant parent: $c^{ch}c^{ch}$ *AM AM*
Strong A parent: *cc am am*
F_1 hybrid: $c^{ch}c$ *AM am*

Genetic breakdown of segregating classes	No.
Mutation white-belly $c^{ch}bAM$	9
Brown non-agouti normal-belly $c^{ch}bam$	3
Albino *cbAM*, *cbam*	4

*c^{ch}—chinchilla, partially dominant to "*c*". Albinism is the homozygous recessive state (*cc*). It alters both black and brown agouti to a light grey agouti but has almost no detectable effect on non-agouti gene.
b—brown. Not used in ratio because it is found in all the animals.
AM—A suspected linkage group found in the mutant, an allele of the agouti series, "*A*" (probably A^w), stands for agouti and "*M*" stands for mutant or white-belly.
am—The recessive form of *AM*, "*a*" stands for non-agouti and "*m*" stands for normal belly.

TABLE II. Results of backcross of the light mutant to albino

Albino		44
Brown non-agouti	17	
Original white-belly mutant	10	27
Total		71
Observed ratio:	44 : 27	
Expected ratio: (1:1)	36 : 36	
Genetic constitution of parents:		
Light mutant: $c^{ch}c$		
Light mutant: $c^{ch}c$		
Albino: *cc*		

Results of Backcross of the Dark Mutant to Albino

Albino	0
Brown non-agouti	17
Original white-belly mutant	25
Total	42
Observed ratio: no albinos	
Expected ratio: no albinos	
Genetic constitution of parents:	
Dark mutant: $c^{ch}c^{ch}$	
Albino: *cc*	

TABLE III. Results of the outcross of the new mutant to the "C" stock (Genetic constitution *AABBCCmm*)

Brown agouti white-belly		687
Brown agouti normal-belly		212
Origianl mutant white-belly		212
Original mutant normal-belly		62
Total		1173
Expected ratio:	(9:3:3:1) 660 : 220 : 220 : 73	
Observed ratio:	687 : 212 : 212 : 62	
Chi square: 3.275	Probability: 0.32	

Genetic constitution of parents and F_1 generation
Mutant: $c^{ch}c^{ch}$*AM AM* (probably A^w)
C stock: *CC Am Am*
F_1: Cc^{ch}*AM Am*

Genetic breakdown of segregating classes	No.
Brown agouti white-belly *CAM*	9
Brown agouti normal-belly *CAm*	3
Original mutant white-belly $c^{ch}AM$	3
Original mutant normal-belly $c^{ch}Am$	1

original mutant frequently occur. They simply are substitutions for white-belly and chinchilla respectively and should be considered as such. In addition the gene for piebald has been dropped from the F_2 results (found in the original mutant) because it behaved in its usual unpredictable manner by not asserting itself phenotypically as frequently as it must have occurred genotypically.

In the outcross to C3H mice (genetic constitution *AABBCC*) however, orange-bellied piebald animals occurred. In the F_2 ratio (without piebald) a *27*:9:9:3:-*12*:4 ratio was obtained, (see Table IV). It is a typical three gene ratio except for the 12:4 section which may be the result of the cumulative effect of chinchilla upon both black and brown agouti. This hypothetical action may cause these classes to combine (nine black white-belly agouti and three brown white-belly agouti is equal to 12; three black normal-belly and one brown normal-belly is equal to four). Thus the segregation is between the white-belly agouti mutant and its normal allele, non-agouti, while the black and brown are acted upon so that both appear phenotypically the same (i.e. not identifiable by gross inspection).

Again considering the orange-belly, one of the first obtained was mated to mice of C3H stock to determine its probable genetic makeup, (see Table V). Of 20 mice obtained in the resulting F_1 generation, all were black white-bellied agouti. Orange-bellied animals have also occurred in several other outcrosses (to C57, C, BrS). It is interesting to note here that orange-bellied animals have not occurred in outcrosses to albino mice.

The first outcross made to a non-agouti, after the cross to Strong A mice, was to the C57 stock (genetic constitution *aabbCC*), (Table VI), which is a black non-agouti normal-bellied stock. It was in this outcross that it was definitely established that the gene for white belly will not occur in a mouse with the non-agouti types, (see Tables VII and VIII).

In the mutant stock, which is being inbred, a great deal of variation has been noted on the ventrum. In the young adult

TABLE IV. Results of the mutant outcrossed to the C3H Strain (*AABBCC*)

Black agouti white-belly	560
Black agouti normal-belly	198
Brown agouti white-belly	192
Brown agouti normal-belly	52
Original mutant white-belly	240
Original mutant normal-belly	81
Total	1323
Observed ratio:	560 : 198 : 192 : 52 : 240 : 81
Expected ratio: (27:9:9:3:12:4)	558 : 186 : 186 : 62 : 248 : 83
Chi square: 2.853 Probability: 0.90	

Genetic constitution of parents and F_1 generation
Mutant: *$c^{ch}c^{ch}bbAM\ AM$* (probably A^W)
C3H stock: *CCBBAm Am*
F_1 generation: *Cc Bb AM Am*

Genetic breakdown of segregating classes	No.
Black agouti white-belly *CBAM*	27
Black agouti normal-belly *CBAm*	9
Brown agouti white-belly *CbAM*	9
Brown agouti normal-belly *CbAm*	3
Original mutant white-belly *$c^{ch}AM$, $c^{ch}bAM$*	12
Original mutant normal-belly *$c^{ch}BAm$, $c^{ch}bAm$*	4

TABLE V. Results of the backcross between orange-bellied mutant and C3H (*AABBCC*)

Black agouti white-belly	20

Genetic constitution of parents:
C3H: *CCBB AmAm*
Orange: *CC (c)BB(b) AM AM*

TABLE VI. Results of the new mutant outcrossed to the C57 strain (*aaBBCC*)

Black agouti white-belly	485
Black non-agouti normal-belly	218
Brown agouti white-belly	169
Brown non-agouti normal-belly	79
Original mutant white-belly	223
Total	1174
Observed ratio:	485 : 218 : 223 : 169 : 79
Expected ratio: (27:12:12:9:4)	495 : 229 : 220 : 165 : 73
Chi square: .797 Probability: 0.99	

Genetic constitution of parents and F_1 generation
Mutant: *$c^{ch}bbAM\ AM$* (probably A^W)
C_{57} strain: *CCBBam am*
F_1 hybrid: *Cc^{ch}BbAM am*

Genetic breakdown of segregating classes	No.
Black agouti white-belly *CBAM*	27
Black non-agouti normal-belly *CBam*, *$c^{ch}Bam$*	12
Brown agouti white-belly *CbAM*	9
Brown non-agouti normal-belly *Cbam*, *$c^{ch}bam$*	4
Original mutant white-belly *$c^{ch}BAM$*, *$c^{ch}bAM$*	12

mouse the ventrum varies from a light cream color to a near yellow color. Of course the ventrum colors with age.

Discussion

The dominance of the mutant dorsum in outcrosses to albinos (Table I and VII) of its recessiveness to possessors of large *C* clearly points to an intermediate allele of *CC* and *cc* as a cause of the modification of the brown agouti dorsum of the mutant. As mentioned by Snell[5], the observation of chinchilla in non-agouti animals is frequently difficult and it is probable that this gene has been carried along in the Brpb stock for a considerable time before the white-belly mutation occurred, possibly as a result of the carcinogen methylcholanthrene.

As for the light and dark mutants found in the F_2 of the albino outcrosses (this phenomenon is not mentioned in the chart for the AKH outcross because of lack of numbers and the repetition) the 2:1 ratio between *Cc* and *CC* is clearly shown (Table II).

As illustrated by the C57 outcross the gene for agouti and the gene for white-belly, if they *are* separate genes, occur simultaneously and must be said to be either closely linked or the same gene. In the Little and Hummel paper[3] it was stated that the white-bellied mutation, which had occured from the DBA stock (similar to Brpb) was A^w. The agouti gene and the white-belly gene were one and the same gene, not closely linked genes. In our experiment there is little or no evidence to support the two gene idea and a good deal of evidence to support the one gene theory. In the first place, it is commonly understood that the A^y, A^w, etc., genes are arranged in an allelic series and that agouti is an effect of all but the two most hypostatic genes of this series. In other words the white belly of A^w does not occur without the presence of the agouti gene. In all noted incidents of A^w occuring as a mutation, in nature or otherwise, the white belly of A^w is epistatic to normal belly; so also is the white belly of this mutant epistatic to normal belly. Thus it seems that the white bellied mutation is in all probability a reverse mutation from *a* to A^w.

The ratio of the C57 outcross (Table VI) is not a classic one and thus needs some explanation. The complete behavior of the chinchilla gene is clearly shown in this outcross because the black, brown, and non-agouti genes are all present. As can be seen by the results, homozygous chinchilla appears to act the same on both black and brown agouti (also white belly); thus one of the classifications is formed (nine black and three brown equal the original mutant white-belly). However, non-agouti is affected very little by chinchilla and the chinchilla normal-bellied (three black : one brown) are placed into the black and brown non-agouti normal-bellied groups (as shown by the table) instead of combining into their own group.

To prove these suppositions two crosses were made. In one, a black non-agouti normal-bellied female was mated to an original mutant to prove that one-fourth of this group (black non-agouti normal-belly) could geneti-

TABLE VII. Results of the new mutant outcrossed to the AKH strain (*ccBBaa*)*

Original mutant white-belly	225	
Non-agouti	89	
Albino	95	
Total	409	
Observed ratio:	225 : 89 : 95	
Expected ratio: (9:4:3)	230 : 76 : 102	
Chi square: 2.602 Probability: 0.45		

Genetic constitution of parents and F_1 generation
Mutant: $c^{ch}c^{ch}AM\ AM\ bb$
AKH: *ccam am BB*
F_1: $c^{ch}c\ AM\ am\ Bb$

Genetic breakdown of segregating classes	No.
Original mutant white-belly $c^{ch}AM$	9
Non-agouti (Comprised of black, brown, and an intermediate due to partial dominance of small *c*) $c^{ch}am$	3
Albino *cAM, cam*	4

*Segregation between Black and Brown is not shown because of lack of necessary data (numbers of animals, etc.).

TABLE VIII. Results of the new mutant outcrossed to mice of the BrS stock

Brown agouti white-belly	226	
Brown non-agouti normal-belly	99	
Original mutant white-belly	82	
Total	407	
Observed ratio:	226 : 99 : 82	
Expected ratio: (9:4:3)	229 : 102 : 76	
Chi square: .492 Probability: 0.92		

Genetic constitution of parents and F_1 generation
Mutant: $c^{ch}c^{ch}bbAMAM$
BrS: *CC bb am am*
F_1: $Cc^{ch}\ AM\ am$

Genetic breakdown of segregating classes:	No.
Brown agouti white-belly *CAM*	9
Brown non-agouti normal-belly *Cam*, $c^{ch}am$	4
Original mutant white-belly $c^{ch}AM$	3

TABLE IX. Results of a cross between an original mutant and a black non-agouti normal-bellied animal from the C57 F_2

Original mutant white-belly	3
Original mutant piebald	1
Total	4

Genetic constitution of parents
Black non-agouti normal-belly: $c^{ch}c^{ch}Bb\ mm\ Ss$
Original mutant white-belly: $c^{ch}c^{ch}bb\ MM\ ss$

cally be chinchilla. (Table IX). In the other mating (Table X) a cross between an F_2 chinchilla from the C3H outcross and a mutant from the original stock (also c^{ch}), it was shown that the modifying gene (chinchilla) affects black as well as brown, as there is little variation in chinchilla white-bellied animals at classification age. However as these animals grow older, a marked difference between black and brown chinchilla becomes evident. But for classification purposes it was necessary to group these two classes together, both in the C57 and C3H outcrosses. As seen from the tables the suppositions used in the C57 and C3H outcrosses are reasonably well supported and the behavior of the chinchilla gene is established (also BrS).

As for the especially interesting problem of the orange-bellied mice, it is established by the data of Table V, that the genetic consititution of these mice consists of the gene for white-belly A^W and the modifying factor piebald (*ss*). But if these were the only genes necessary, the mutant stock itself should be orange-bellied. However, it has been noted that all outcrosses producing orange-bellied animals have been under the influence of the dominant gene *CC* for color. If this is true, then orange-bellied animals should not show up in outcrosses to albinos, as the mutant itself does not have large *C* but c^{ch}. This is exactly what happens; orange occurs in the crosses to C, C57, BrS, and C3H but does *not* occur in the crosses to AKH and Strong A mice. Thus it seems that the orange color is due to the cumulative effect of three genes: dominant A^W, recessive *pb* (*s*), and dominant *C*.

This is still another proof of the hypothesis that the white-bellied mutation is A^W. In several papers, the color orange is associated with a member of the agouti series. In Little's paper[4] an orange-bellied stock occurs through selective breeding but in our experiment they occur spontaneously as a member of a genetic ratio. Also in Little's experiment the orange color changed to the white of the A^W mouse with age, but the mice in this experiment do quite the opposite, they become more vividly orange with age. Whether the orange is due to different modifying genes in the respective cases is a matter of question. However, the fact remains; the orange color in the house mouse seems to be dependent upon a member of the agouti series for a part of its phenotypic expression.

One of the tasks of the future will be to prove more conclusively that the white bellied mutation is A^W. This will be done by mating the mutant to the various members of the agouti series and studying the dominance and segregation of these various crosses.

TABLE X. Results of a cross between a chinchilla white-belly (from the F_2 generation of the C3H outcross) and BrS to establish that three-quarters of F_2 chinchillas could be black in the C3H and C57 outcross

Black agouti white-belly	19
Black agouti normal-belly	14
Brown agouti white-belly	10
Brown agouti normal-belly	8
Total	51

Genetic constitution of parents
F_2 chinchilla: $c^{ch}c^{ch}Bb\ Mm\ AA$
BrS: *CCbbmmaa*

Summary

The highly probable reverse mutation of *a* to A^W, complicated by the addition of the chinchilla gene, which had been carried by the Brpb stock before the mutation, has its interesting aspects, i.e., it has been shown that a mouse having A^W with c^{ch} in the homozygous state produces a white-bellied agouti mouse whereas with the dominant allele of chinchilla (*C*) the orange-belly agouti appears. ($A^Wc^{ch}c^{ch}$ = white belly agouti; A^WCC = orange-belly agouti.) It would be interesting to find out if each allele of the albino series (C, c^t, c^{ch}, c^e, c) affects the white-belly mutant differently, perhaps in the degree of yellow (white-to-orange) on the ventrum. This mutation to A^W, though not the first mutation to A^W, seems to present both an interesting example of a rare reverse mutation and a study of irregular genetic ratios due to modifying factors present in the mutant stock.

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